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ANNALS
OF THE
NEW YORK ACADEMY
OF SCIENCES

VOLUME XLV



NEW YORK
PUBLISHED BY THE ACADEMY
1943-1944

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THE RELATION BETWEEN CENTRIOLE AND CENTROMERE IN ATYPICAL SPERMATO- GENESIS OF VIVIPARID SNAILS*

By

ARTHUR W. POLLISTER† AND PRISCILLA F. POLLISTER‡

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* Awarded an Honorable Mention in the A. Cressy Morrison Prize competition in 1942. Publication made possible through grants from the Centennial Fund and the Ralph Winfred Tower Memorial Fund.

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INTRODUCTION

In most genera of prosobranchiate snails two sorts of spermatozoa are formed. One of these, called typical or eupyrene, is a cell of normal molluscan type, with a single axial filament and a single flagellum. The other, the atypical, is usually much larger, and is very abnormal. There are many flagella and axial filaments; and, in the mature spermatozoon, the chromatin is either completely absent (apyrene condition) or is greatly reduced (oligopyrene condition). Snails of the Family Viviparidae produce atypical spermatozoa that are oligopyrene. Meves (1902) showed, in the European *Viviparus* (*Paludina*) *viviparus*, that the oligopyrene spermatozoa have many axial filaments because there are many centrioles to function as blepharoplasts instead of the single centriole of a typical spermatozoon, and he found that the reduced amount of chromatin was a result of the fact that most of the chromosomes degenerated before early spermiogenesis.

Meves and all subsequent workers have assumed that the increased number of centrioles is a consequence of a series of divisions of the two normal centrioles independent of either the cell or the nucleus. This multiplication has been supposed to occur during the growth period of the atypical spermatocytes, when each of the two centrioles grows to a large sphere that later breaks up into a number of centrioles. The case has always been of great interest to students of centrioles, since it is apparently the least questionable example of multiplication of centrioles independent of other parts of the cell, and it has been invoked as support for the Henneguy-Lenhossek theory that basal bodies of cilia arise by a process of centriole multiplication.

From a more modern viewpoint it seems possible that the presence of the extra centrioles in atypical spermatogenesis may have quite a different explanation—one that also accounts for the abnormal meiotic behavior and the ultimate degeneration of so many of the chromosomes. In 1936, Schrader pointed out that similarity in size, staining reactions, and appearance in living cells suggest that the centriole and the spindle fiber attachment body (his kinetochore, or specifically the spindle spherule) are related to one another. Darlington (1937), on grounds that were largely theoretical, has urged the view that the centriole and the spindle fiber attachment body (his centromere) are identical bodies, which normally show differences in behavior because one is in the cytoplasm and the other is in the nucleus as part of a chromosome. If centriole and centromere are the same, then it is conceivable that a centromere could become detached from its chromo-

some, become located in the cytoplasm, and there act as a centriole. In such a case the chromosome, lacking a centromere, would be expected to behave as does an acentric chromosome fragment. It should be incapable of orienting correctly on the metaphase plate and, lacking a spindle fiber, it should be unable to proceed accurately to one pole of the spindle, though it might be able to reach a position in the vicinity of the pole (Carlson, 1938). An examination of Meves' paper on *Viviparus viviparus* showed us that the abnormal chromosomes did, in fact, behave as if acentric; and this suggested the possibility that the extra centrioles of atypical spermatogenesis were not, as had hitherto been believed, the result of multiplication of the two normal centrioles, but were actually centromeres that had become detached from chromosomes. If this were so, then the number of extra centrioles should bear a definite numerical relation to the chromosomes that behave as if acentric. Meves did not give the exact data necessary to prove this point. Our preliminary examination of a form that was later identified as the Japanese *Viviparus malleatus* fully supported the idea that the extra centrioles are detached centromeres. For it was found that there is one extra centriole for every chromosome that behaves as if acentric (Pollister, A. W., 1939). The present report is a detailed study of *Viviparus malleatus*, and also includes confirmatory data from three American genera of the Family Viviparidae.*

MATERIAL AND METHODS

Viviparus malleatus Reeve (Viviparidae, Prosobranchiata) is a Japanese form that has been introduced into many localities in the United States. Our specimens were purchased from aquarium supply dealers, notably William Tricker of Saddle River, New Jersey. Testes are in good condition from May to the end of October. *Lioplax subcarinata* Say was collected in the Delaware River at Delanco, New Jersey, in September. Study of *Campeloma ponderosum* (Lea) was from the testis of a single male collected beneath the bridge over the Coosa River at Wetumpka, Alabama, in late May. In several hundred specimens of various *Campeloma* species from Wisconsin, New York, New Jersey, and Maine, we have found no males. A single female specimen of *Tulotoma magnifica* Conrad was found in June in the shoals of the Coosa River near the farm of Mr. E. A. Cobb of Wilsonville, Alabama. In the following October, Mr. Cobb very kindly sent us a large collec-

* We are deeply indebted to Doctor Sally Hughes-Schnader for reading critically the entire manuscript and offering many constructive suggestions.

tion that included many males. We are indebted to Doctor H. A. Pilsbry of The Philadelphia Academy of Sciences for identification of these forms.

Viviparidae, like most operculate snails, will stand shipment for long distances, especially if packed in wet moss and sent during the cooler months. In all the members of this family the genital duct runs through the right tentacle of the male. In all American Viviparidae the right tentacle is thicker than the left and is blunt rather than pointed. In the Japanese *V. malleatus* the two tentacles are nearly the same size and shape, but the right tentacle of the male is carried in a curled-over position. The lower lobe of the testis is always quite distinct in mature animals, and it is easily dissected out as an entire organ. If the dissection is made in the fluid of the animal (mantle cavity fluid?) the spermatozoa are very active. This is not so if the dissection is made in Ringer's fluid. The former method was used in all dissections for cytological study.

Material was fixed in the following fluids: Hermann's, Flemming's, San Felice's, Helly's, Bouin's, Champy's or Benda's—as a rule, for 24 hours. The centrioles are well shown, and rather specifically stained by Benda's method for mitochondria. This is, however, a capricious technique, and it does not yield preparations that are useful for chromosome studies. Material fixed over night in Hermann's fluid, bleached for 30 minutes in one part of commercial hydrogen peroxide solution to four parts of 70 per cent alcohol, and then stained with iron hematoxylin gives nearly as good slides for centriole counts, and certainly much the best fixation of chromosomes. Newton's gentian violet is a much better chromosome stain. The fairly simple routine followed in the later studies was to cut one-half a block of tissue of Hermann-fixed material at 6 microns, the remainder at 12 microns. The thinner sections were stained with iron hematoxylin, the thicker with gentian violet. Each stain was preceded by the peroxide bleach. Smears seem to be no more useful than thick sections. The condensed chromosomes are very difficult to fix. They are apparently very fluid, for they often appear as spheres even in unfixed cells. Many of our fixed preparations, however, show orthodox V-shaped chromosomes, and we feel safe in assuming therefore that the other shapes are distortions due to the highly fluid nature of the chromosomes.

The centriole counts involve very exacting microscopy. Most of them were made with a Zeiss 60X, N. A. 1.4 objective, ocular magnification to give 1350X, illumination by Zeiss Pancratic Condenser, and

with a Zeiss colored glass filter that is very much like the Wratten No. 59. Observations were made in a completely darkened room.

We have not found it possible to demonstrate the essential part of the centromere, the spindle spherule, in the chromosomes. This is perhaps not surprising, for it is barely large enough to be detected even in the largest animal chromosomes, those of Amphibia (Schrader, 1936, 1941).

OBSERVATIONS

In the general features of meiosis and spermiogenesis, all the Viviparidae we have studied agree with Meves' findings on *Viviparus (Paludina) viviparus*. We shall therefore treat very briefly the facts he dealt with so fully, and devote our attention chiefly to points that were, quite understandably in the state of cytology of 1902, ignored by Meves. The complete course of spermatogenesis will be outlined in *Viviparus malleatus*, with occasional reference to features that are more strikingly shown in the other forms.

Chromosomes of the Typical Line

We have not been able to distinguish two types of spermatogonia corresponding to the two types of spermatocytes, the typical and the atypical. All small spermatogonia have a finely granular nucleus with two nucleoli (FIGURE 1).^{*} There are two centrioles in the idiozomal region, some distance from the nucleus (FIGURE 2).[†] In prophase of a late spermatogonial division, the chromosomes take up a position evenly dispersed just inside the nuclear membrane, so that when the center of the nucleus is in focus it appears empty (FIGURES 3 and 4). At this time the nucleoli have disappeared, and the two centrioles are close to the nuclear membrane. At prometaphase, the chromosomes clump together as soon as the nuclear membrane breaks down; and it is from this clump that they move to the metaphase plate. The diploid number of chromosomes is 18 (FIGURE 5). In well-fixed preparations it is clear that these are V's, with equal or subequal arms.

^{*} Each nucleolus consists of a thin layer of chromatin surrounding a spherical mass of what appears to be a typical plasmosome material. With Auerbach's acid fuchsin and methyl green the plasmosomes are red, in contrast with the green chromatin. The nucleoli are red in eosin-azur stain of Nocht-Maximow (used as directed in Romeis, B., 1932, *Taschenbuch der Mikroskopischen Technik*, R. Oldenbourg, Berlin, page 391) after fixation in Helly's fluid. With Benda's alizarin-crystal violet method the nucleolus is colored brownish red by the alizarin (Kahlbaum's). After fixation in Champy's fluid the nucleolus is much more tenacious of hematoxylin than is the chromatin—so that the latter may be completely destained, leaving the plasmosome completely black. The plasmosome stains green in orcein-fast green smears. It is negative to the Feulgen nuclear reaction, and it is not stained by Newton's gentian violet method.

[†] These FIGURE numbers refer to the individual FIGURES ON PLATES 1-5 at end of paper. The line-out illustrations are indicated as TEXT FIGURES.

There is nothing unusual about the meiotic phenomena in the typical line. Most of the growth takes place prior to leptotene (FIGURES 6, 7 and 8). As the slender leptotene threads approach synapsis, it is apparent that their ends are oriented toward the idiozomal pole of the nucleus (FIGURE 8). This bouquet orientation is very marked at pachytene (FIGURE 9), and the ends of the chromosomes are then all in a small area beneath the nuclear membrane adjacent to the idiozomal region (FIGURES 9-10). It is actually during this period of movement to the bouquet position, that the chromosomes first show a tendency to take position on the nuclear membrane (FIGURE 11)—not at the much later period of diakinesis, as has often been described in other forms. This, however, is somewhat obscured by the fact that the loops of shorter chromosomes pass across the center of the nucleus (FIGURES 10 and 11). The typical diakinetic arrangement, with the chromosomes evenly spaced just beneath the nuclear membrane (FIGURE 13), follows immediately the late pachytene; for one may find these two stages, and no others, in the same group of cells. This equal spacing of diakinesis seems to be a consequence of the release of the ends of the chromosomes from their attachment to the idiozomal pole of the nucleus.

The two nucleoli increase in size during the growth period, then dwindle during leptotene. They fuse to a single nucleolus when synapsis occurs (FIGURES 9 and 12). This single nucleolus, associated with a region near the end of one of the larger chromosomes, persists till nearly the end of pachytene. It has invariably disappeared by diakinesis.

At the conclusion of the usual tetrad condensation of diakinesis (FIGURES 14 and 15), the nuclear membrane breaks down and the tetrads become crowded together into a compact mass (FIGURE 16), as were the chromosomes of the spermatogonial prometaphase. Between the centrioles, at opposite sides of the nucleus and the mass of chromosomes, large fibers develop (FIGURE 16) and, when the tetrads move into the metaphase plate position with these heavy fibers as the half-spindle components (FIGURE 17), it is evident that there are 9 tetrads (FIGURE 18). During interkinesis the nucleus becomes diffuse.

When the chromosomes condense for the second division, they again become evenly distributed on the nuclear periphery (FIGURES 20 and 21), as in spermatogonial divisions and diakinesis, and they likewise again become aggregated into a clump at prometaphase (FIGURE 22). After the second division has run its course (FIGURES 23 and 24), the

nucleus of each spermatid enters upon a series of internal changes (FIGURES 25 and 26) that culminates in the compact nucleus of the mature spermatozoon. At first, while the spindle remnant is yet large, the chromosomes, still compact, take positions on the nuclear membrane (FIGURE 25). Each chromosome next appears to flatten and spread out along the nuclear surface; and the separate chromosomes, now irregular in outline, come into contact to form a coarse two-dimensional reticulum. There follows a very conspicuous stage when this reticulum is in the form of a slightly more than hemispherical shell, and the remainder of the nucleus then appears as an empty space bounded by the nuclear membrane (FIGURE 26). The chromatin is now less tenacious of hematoxylin than the centrioles, so that, in preparations suitable for study of the latter, the entire nucleus appears as an empty vesicle. Up to this time the nucleus has been nearly spherical, but now its posterior region, near the center of which lies the proximal centriole, becomes flattened and then actually invaginated.

Centrioles of the Typical Line

The centrioles remain some distance from the nucleus, as in the spermatogonia, during the early growth stages of the typical spermatocytes. In leptotene, they move to positions adjacent to one another and close to the nuclear membrane (FIGURE 8). Here the two centrioles remain until late diakinesis, when they move apart along the nuclear membrane (FIGURE 15). At this time, an aster first appears about each centriole. Each centriole is first clearly divided by early telophase of the first division (FIGURE 19). After telophase the sister centrioles are located close together near the center of the main mass of cytoplasm (FIGURE 20), and here they remain throughout interkinesis. As the chromosomes condense in the second prophase the centrioles first move to the nuclear membrane (FIGURE 21); then move apart along the nuclear membrane, and an aster develops about each centriole (FIGURE 22). During the development of the division figure, the centrioles become located nearly on the cell periphery on opposite sides of the cell and they remain in this peripheral position during the second division (FIGURES 23-25). In early telophase, while the spindle remnant is still conspicuous, a flagellum grows out from each centriole (FIGURE 25). As the chromatin takes on the hemispherical shell pattern, the centriole divides into a distal part on the cell periphery, and a proximal centriole on the nuclear membrane, the two being connected by an axial filament (FIGURE 26). Throughout spermiogenesis the

proximal centriole remains close to the nuclear membrane. The distal is on the cell periphery as long as it can be seen. The nuclear invagination and the cell elongation of later spermiogenesis thus involve a progressive lengthening of the axial filament. It should be added that the proximal centriole remains constant in size, while the distal one progressively dwindles until it cannot be seen during later spermiogenesis.

Chromosomes of the Atypical Line

An early atypical spermatocyte is shown in **FIGURE 27**. The mature atypical spermatocyte is several times the volume of that of the typical line. This is entirely due to a difference in cytoplasmic growth, for there is no measurable difference in nuclear size. (Compare e.g., **FIGURES 13** and **38**.) At no time is there any bouquet orientation of the chromosomes; and there are no appearances that suggest synaptic phenomena in the atypical nucleus. This absence of the nuclear phenomena peculiar to meiosis makes it difficult to compare the stages in the two lines. We have assumed that the disappearance of the nucleolus and the arrangement of chromosomes in an even spacing on the nuclear periphery occur at approximately the same stage in both lines—one that is comparable with the beginning of diakinesis in the typical spermatocytes. In both lines, this nuclear stage is reached only after cytoplasmic growth has ceased. The most conspicuous change in cytoplasmic elements in the typical line is the movement of the centrioles to the nucleus in early leptotene. In the atypical line this does not occur until much later—not until early diakinesis as defined by the nuclear phenomena. This delay in the approach of the centriole to the nucleus seems to be the first detectable difference other than cytoplasmic growth between the two lines.

The slender chromosomal threads of the early atypical spermatocytes gradually thicken as cell growth proceeds (**FIGURES 29, 31, 32** and **36**). In the well-fixed nuclei these threads appear at first much like those of the typical leptotene. When the chromosomes first assume the orientation on the nuclear periphery they are about as thick as the threads of unpaired regions of typical early pachytene (compare **FIGURES 32** and **11**). During diakinesis, each of the chromosomes in the atypical nucleus is seen dividing into two slenderer threads (**FIGURE 37**).^{*} This separation leads to complete isolation of the two sister

^{*} In the preliminary paper (Pollister, 1930), one of these pairs of disjoining chromatids was misinterpreted as a tetrad.

threads; and, in later stages of chromosome condensation, one often sees two chromosomes of identical size and shape adjacent to one another. When the products of this division become evenly dispersed on the nuclear periphery in late diakinesis, they may be counted fairly accurately (FIGURE 38). There are close to 36, four times the haploid number, and 36 is the number that can be determined with certainty later (see below and FIGURES 45 and 47). From the absence of synaptic phenomena and from the separation of the elements of pairs of chromosomes seen in early diakinesis, we conclude that this number of chromosomes, 36, results from the separation of sister chromatids of unsynapsed chromosomes. Each thus corresponds to one of the chromatids of the typical spermatocyte at late diakinesis. The same number of chromatids are present in each line: in the typical spermatocytes, they are in nine groups of four; in the atypical line, all 36 are seen as separate bodies.

The breakdown of the nuclear membrane is followed by a clumping of the chromatids (FIGURE 39) in the manner also seen in prometaphase of spermatogonia and in typical spermatocytes. The next events, however, bear very little resemblance to normal metaphase. There is some hint that a few chromosomes form half-spindle components (FIGURES 40 and 41) and there is possibly an approximate metaphase orientation of a few chromosomes (FIGURE 42), but most of the 36 chromosomes move to the nearer spindle pole without at any time being on a metaphase plate (FIGURES 44-46). Eventually, many of the chromosomes tend to form two groups, one near either pole (FIGURES 44 and 45). Since it is a poleward movement of chromosomes, we shall, purely for convenience, refer to this stage as anaphase. For most of the chromosomes, the resemblance to normal anaphase lies only in the poleward migration. No half-spindle component is attached to them, and their orientation with respect to the direction of movement appears random, within the limits that a chromosome arm cannot move otherwise than parallel with an adjacent astral ray or spindle fiber. A polar view of such an anaphase of an atypical spermatocyte (FIGURE 43) is a striking contrast to the regularity of arrangement of a normal group of V-chromosomes as they approach the pole with their apices all pointing in toward a common center.* The regularity of arrangement of a

* The acentric chromatids of *Viviparus* are two-armed and characteristically appear V-shaped. This V shape has no relation to the orientation of the chromosomes with respect to the spindle pole, indeed, it is even seen in diakinesis. This seems at first somewhat surprising in view of the fact that the V shape of a normal chromosome appears generally to be a consequence of the pull of the localized centromere on the more or less passive remainder of the chromosome. Perhaps the V shape of the acentric chromatids of *Viviparidae* can be explained by the assumption that the removal of the centromeric mass has left this part of the chromosome as a point of weakness, much more flexible than the two stiff arms, which are thus free to assume any angle with respect

normal set of V-chromosomes is, of course, due to the fact that the apically located centromeres lead the way to the poles; and the contrasting irregularity of orientation of the V's of the atypical anaphase suggests that, in most of these chromatids, the centromere either is missing or is not functioning normally. From evidence in the second division and from our theoretic analysis, we shall find reason for believing that this complement of 36 chromatids contains 4 that have normal centromeres. There is some further evidence from the anaphase of the first division for the presence of these chromosomes with centromeres, or centric chromatids; for one frequently sees at a pole two chromosomes that have well-developed, half-spindle components (FIGURE 48). These are oriented toward the pole in the normal fashion. In smears (FIGURE 47) or in thick sections (FIGURE 45), one can count the full complement of 36 chromatids in the atypical first division. The numbers around the two poles are rarely the same, but the difference is not more than a few chromosomes. At the time when the spindle is elongating, just before cell constriction, many of the chromosomes leave their positions at the pole; and, moving within the spindle and parallel with its fibers, these chromosomes migrate to positions approximately midway between the spindle poles. This is most striking in *Lioplax subcarinata*, in which eventually nearly all of the chromatids are in a rather narrow plane midway between the two poles (FIGURES 49-51). The cell constriction pushes them but a short distance away from the equator. Our seriation of these stages is certain. For these chromosomes are still near the cleavage furrow when they undergo their telophase alterations. Along with stages like FIGURE 51, one finds others well advanced into telophase.

At the telophase of the first division a small nucleus of normal appearance develops in each secondary spermatocyte (FIGURES 53 and 55). Occasionally there are two small nuclei instead. We have never seen more than two. These normal nuclei are always near the pole of the division figure. Hence they must have arisen from chromosomes that did not participate in the counterpole movement of late anaphase. This is an indication that these chromosomes that form the normal nucleus are centric chromatids and thus are held at the poles as normal chromosomes always are when the telophasic changes begin.

to one another. The chromosome is like two sticks attached by a ball-and-socket joint. V's of various angles would be expected if these arms are thus free to rotate—and this is, in fact, what we see in the acentric chromatids at diakinesis and at division I. It also appears that frequently the two arms are parallel, especially where the chromosomes are compelled to conform to the linear structure of the spindle as they move equatorward at late anaphase. Least common, as one would expect, are cases where the two arms are in line. It is possible, alternatively, that the V shape of the chromatids is a sort of holdover from the anaphase of the last spermatogonial division.

The chromosomes that are not included in the normal nuclei do not fuse together. In the few cases where we have counted these, e.g. FIGURE 53, we have found there are 32 in the two sister secondary spermatocytes. The small normal nucleus, then, contained 4 chromatids (36 less 32)—a number that agrees with the number of centric, or normal, chromatids of division II. Each of the chromosomes not taken into the normal nucleus becomes a peculiar vesicular body, in which the chromatic material is concentrated in one hemisphere (FIGURES 54, 55 and 58). At first, the chromatin of these vesiculated chromosomes stains, by every method we have used, like normal chromatin. The vesicular form is very evident in freshly teased cells. Under these conditions, the chromatic part of the vesicle appears much more highly refringent than does normal chromatin; but this may well be a consequence of the lenticular shape of the former. At later stages, certain technical methods reveal differences between the vesicular and normal chromosomes. In the second division, they take the alizarin strongly, while the normal chromosome stains blue with crystal violet. They are much more intensely stained with azur II. The acid fuchsin of Auerbach's stain colors the vesicular chromosomes, while the normal ones are colored with the methyl green.

Once the chromosomes have become vesicular, they are incapable of going through a normal chromosome cycle. After the first division, they undergo no conspicuous changes of form. Throughout the course of the second division they are passive, showing no tendency to respond in any specific manner to the mitotic forces that are then in action (FIGURES 61–65). Their disappearance begins soon after telophase of the second division and is soon completed (FIGURES 66, 67, 68 and 69). (The vesicles in FIGURES 71, 72 and 73 are acroblasts, not chromosomes.)

There is no metaphase plate in the second division. The poleward anaphase movement involves but two chromosomes in each secondary spermatocyte. A spindle is established across the cell, with these two normal chromosomes at its poles (FIGURES 61–65). The second division in the Japanese snail, *Viviparus malleatus*, is unequal (FIGURES 63, 65, and 69). (It is not so in the European *Viviparus viviparus* or in American *Viviparidae*.) The contents of the two unequal sister spermatids of *Viviparus malleatus* are different. The division plane is so oriented that all the abnormal degenerating chromosomes and a single normal chromosome become included in the larger cell, while the smaller cell receives only a single normal chromosome (FIGURES 63 and

65). (Very exceptionally one or two abnormal chromosomes are included in the smaller cell.) The sister spermatids are further distinguishable by the fact that nearly all the mitochondrial granules become included in the smaller cell; so that, in a mitochondrial preparation, the smaller sisters can be at once distinguished by their darker stain (FIGURES 65, 69, 70, 72, 74, 76 and 78) from the larger spermatids (FIGURES 65, 69, 71, 73, 75, 77 and 79).

At telophase of division II, a small nucleus forms from each normal chromosome. This goes through a series of changes in form and chromaticity which parallel those in the normal spermiogenesis

The smaller spermatid becomes a small, oligopyrene, atypical, spermatozoon, with cytoplasm that is granular from its mitochondrial content (FIGURE 78). The larger spermatid forms a larger spermatozoon. Its cytoplasm is basophilic, probably because of the acid material of the dissolved abnormal chromosomes, but it is not granular (FIGURE 79). Obviously these two types of spermatozoa must occur in equal number. Morita (1932) has shown by ample data that all Japanese Viviparidae, including *V. malleatus*, form atypical spermatozoa in two size classes in equal number

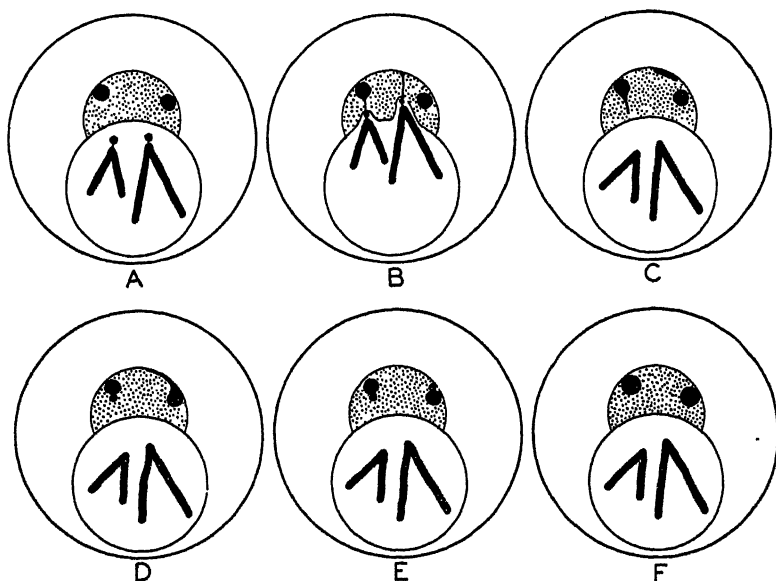
In summary of the chromosome behavior in the atypical spermatogenesis of *Viviparus malleatus*: there is no synapsis; the sister chromatids separate during diakinesis, so that throughout the maturation divisions we have to deal with 36, 4N, chromatids in place of the 9 (N) tetrads of the normal or typical spermatogenesis. Among these 36 there appear to be 4 normal chromatids. These go to the poles normally at both divisions. One of them enters each atypical spermatid and, by a normal series of changes, becomes the nucleus of the atypical spermatozoon. The other 32 chromatids become abnormal at telophase of the first division and ultimately degenerate and disappear. They do not go to the poles at the second division. Though they do so at the first division, their lack of accurate anaphasic orientation and their failure to remain at the poles show that this is not a normal anaphase movement regulated by the centromere. The details of chromosome behavior during the maturation divisions show that, of the total complement of 36 chromatids, 4 are normal and centric; 32 are abnormal and are acentric.

Centrioles of the Atypical Line

In the idiozomal region of the early atypical spermatocytes (FIGURE 28) there are two bodies slightly larger than the centrioles of the typ-

ical line. These two larger bodies are equivalent to much more than the two centrioles of the typical spermatocytes and we shall accordingly refer to them, not as centrioles, but as centriole spheres or, more conveniently, *centriospheres*. During the growth period of the spermatocytes each centriosphere also grows, eventually reaching a diameter of somewhat over one micron (FIGURES 30-32 and 34). At no time, is there any measurable difference in size of the two centriospheres in a cell. The centriosphere growth begins before the nucleoli dwindle in size, and it is completed at about the time the nucleolar material disappears. Assuming that the nucleolar history is an index of the meiotic chromosome cycle in both typical and atypical line, it appears that the centriosphere growth begins at a stage equivalent to early leptotene and is completed before the end of pachytene (see p. 7). In view of the very strong indirect evidence that this growth is a result of addition of centromere material from the chromosome (see below) it is extremely interesting that there is also direct evidence of this occurrence. In many cells one or two lines of stainable material run from the nucleus through the idiozomal mass to its surface. Frequently, the idiozomal surface of the nucleus has several small lobes protruding into the idiozome; and, in such cells, the stainable filaments begin at the tips of the lobes (FIGURE 33). In other cases, there is no lobe where the stainable line leaves the nucleus (FIGURE 34). Each stainable filament from the nucleus terminates at the surface of the idiozome. The centriospheres also lie on the surface of the idiozome; and it is of great interest that, in certain cases, the line of stainable material actually ends in one of the centriospheres (FIGURES 33 and 34). Such an appearance may be readily interpreted as an interchange of material between the nucleus and the centriosphere. More commonly the line of stainable material that runs to the centriosphere originates from another point on the idiozomal surface, instead of directly from the nucleus (FIGURE 33, and compares Meves' FIGURES 63 and 64). It seems then that there are two methods by which the stainable material is transferred from nucleus to centriosphere: it may flow directly to the latter; or it may accumulate first on the surface of the idiozome and later flow from that point to the centriosphere. All of these appearances which suggest outflow of material from the nucleus and its accumulation in the centriosphere are sporadic. Hence the growth of the centriosphere is probably not the result of a continuous addition of nuclear material, but involves a series of small outflows. The common occurrence of a small lobe on the centriosphere

(FIGURE 35) may indeed represent the accumulation of a single one of these nuclear contributions on the centriosphere surface just before the incorporation of the former into the main mass of the centriosphere. It is therefore highly significant that these centriosphere lobes are approximately the same size as the individual centrioles that will later appear within the centriosphere (p. 6). To anticipate somewhat, we shall later find strong reasons for a belief that the growth of the centriosphere is due to the addition of the masses of 8 centromeres to each of the two centrioles normally present. From the cytological picture, it may be suggested that the escape of the centromeres from their chromosomes proceeds somewhat as shown in TEXT FIGURE 1.



TEXT FIGURE 1. Scheme of growth of the centriosphere by addition of centromeres.

A. Centriospheres (black spheres) in idiozomic region (stippled). In the nucleus are two chromosomes, large and small, that are to be the next to lose their centromeres. The remainder of the chromosomes, not shown here, would include some that had already lost centromeres, some that would later lose them, and others that would remain centric throughout spermatogenesis.

B. There are two nuclear protrusions or lobes; and through each of these the substance of one of the centromeres is flowing across the idiozomic region to its outer surface. The centromeric substance from the small chromosome (left) is flowing directly to a centriosphere (compare PLATE 2, FIGURE 33); that from the large chromosome (right) is accumulating on the surface of the idiozome.

C. The flow of centromeric material from the nucleus is complete; the nuclear protrusions are withdrawn; and the two chromosomes are now acentric. The material of the centromere of the large chromosome has spread out on the surface of the idiozome; that from the small chromosome is accumulating at the surface of the centriosphere on the left.

D. Centromeric material from the large chromosome is now flowing from the region on the idiozomal surface where it first accumulated to the centriosphere on the right (PLATE 2, FIGURE 33); the centromeric material from the small chromosome has accumulated on the surface of the left centriosphere as a small lobe.

E. The accumulation of the centromeric material on the centriosphere is completed; and each centriosphere has a lobe that consists of the mass of one centromere (compare PLATE 2, FIGURE 35).

F. Each centriosphere lobe has been drawn into the whole mass, which is thus again spherical. Each centriosphere has now grown by the volume of one centromere.

The centrospheres stain heavily with hematoxylin, as do the centrioles of typical spermatocytes. They are not stained by the Feulgen nuclear reaction, or by Auerbach's acid fuchsin-methyl green, or by Newton's gentian violet. With Benda's mitochondrial stain the centrospheres are strongly alizarinophilic, in which reaction they resemble the chromosomes and differ from the typical centrioles, which stain only with the crystal violet.

At the beginning of diakinesis, the two centrospheres take positions near the nucleus, and an aster develops around each sphere. At this time, the appearance of the centrosphere changes. It is no longer a uniform spherical mass, but instead, as Meves described it, is mulberry-like—quite evidently made up of a number of tightly packed small granules (FIGURE 36). Also its staining characteristics change suddenly. The small granules are not alizarinophilic but are heavily stainable with crystal violet, like typical centrioles. In their staining reactions, size, and behavior, the granules that appear within each centrosphere are indistinguishable from centrioles of the typical spermatocytes; and we shall call them centrioles from now on. Their appearance terminates the history of the centrosphere. Because of their compact arrangement it is obviously impossible, at first, to determine the number of centrioles in each centrosphere. An occasional somewhat looser grouping at a later stage, however, permits a sure count of 9 (FIGURE 41, left pole). This is the number one would expect from counts of centrioles in spermatids. From the fact that the two centrospheres are the same size, it may be inferred that an equal number of centrioles arises from each.

In late diakinesis, the two masses of centrioles take up symmetrical positions on opposite sides of the idiozomal surface of the nucleus; the asters enlarge; and a spindle develops (FIGURES 39 and 40). These phenomena closely parallel the occurrences at the end of the first meiotic prophase of the typical line, the group of centrioles from a single centrosphere behaving like a single centriole of the typical spermatocyte. The elongation of the spindle in the first division brings the two clumps of centrioles close to the cell periphery for a time (FIGURES 44 and 48).

As the asters fade and the daughter cells round up at cell cleavage, the centriole clumps remain at the spindle ends; and, since spindle elongation has ceased, each thus becomes drawn into a position somewhat poleward from the center of the daughter cell (FIGURE 52). This coincides with the location of the typical centrioles at this stage (FIG-

URE 19). During late telophase of division I, the number of centrioles in each group increases until it is finally approximately doubled (FIGURES 52, 54, and 55), and it thus appears that each centriole divides at about the time when the single centriole of a typical primary spermatocytes does so (see p. 8). Slightly later the compact centriole clump breaks up (FIGURES 54 and 55). As the centrioles move apart, a small aster appears around each (FIGURE 56). This last is most noteworthy, for it shows that each centriole, whatever its origin, now possesses one of the two fundamental properties of centrioles—that of causing the surrounding cytoplasm to become organized into an aster. By the time the spindle remnant has nearly disappeared, the centrioles have taken up individual positions on the periphery of the cell (FIGURE 57). Next, the individual centrioles, still on the cell periphery, collect in small groups, of two to four. Around each of these groups is a single aster which is larger than around each single centriole of the immediately preceding stage (FIGURE 58). This aggregation of centrioles continues, until finally there are but two groups on opposite sides of the cell (FIGURE 59). Each group is the focus of a large aster (FIGURE 60). The number of centrioles is not equal in the two groups. In many cases, it is quite evident that one group—that to be included in the larger daughter spermatid—consists of many more centrioles than the other (FIGURES 62 and 69).

The centrioles remain just beneath the cell surface during the second telophase (FIGURES 65 and 69). A flagellum grows out from each centriole; and, slightly later, each centriole divides into proximal and distal parts which are connected by an axial filament (FIGURES 72 and 73). This demonstrates that each centriole of the atypical spermatid possesses the very distinctive centriolar property of producing a flagellum. In this, and in the later division into two parts, no differences are detectable among the individual centrioles of the atypical spermatids.

In summary of the centriole behavior: in place of the two centrioles of normal cells, two large centrospheres, equal in size, develop during the growth period of the atypical spermatocytes; the growth of the centrospheres appears to be the result of a series of additions of material from the nucleus; eventually, each centrosphere becomes resolved into a group of centrioles. All the centrioles are alike and all resemble the centrioles of typical spermatocytes: (a) in size, (b) in staining reactions, (c) in taking positions at the spindle poles, (d) in details of centriole movements in prophases of the two divisions, (e) in the ability to organize an aster, (f) in causing a flagellum to develop, (g)

in dividing into proximal and distal centrioles that remain connected by an axial filament, and (h) in dividing late in the first meiotic division.

The series (a-h) just enumerated includes the sum of the cytologically detectable features of the structure and behavior of centrioles. Whatever their origin, once they appear within the centrosphere, the many centrioles of the atypical spermatocytes of *Viviparus malleatus* behave in every way like normal centrioles—like those of the typical normal cells of the same testis and like those of male germ cells in general. The bizarre and abnormal aspects of atypical spermatogenesis are due to the larger number of centrioles and to the peculiar behavior of many of the chromosomes. From strict standpoint of the behavior of the individual centriole, atypical spermatogenesis is a perfectly normal manifestation of the reaction of the centriole to the special cellular conditions of meiosis and spermiogenesis. That all the centrioles of the atypical cells behave as typical normal centrioles is especially remarkable, in view of the fact, as will appear from the analysis below, that they appear to be really of two sorts—the true centrioles, and extra ones that have arisen from centromeres.

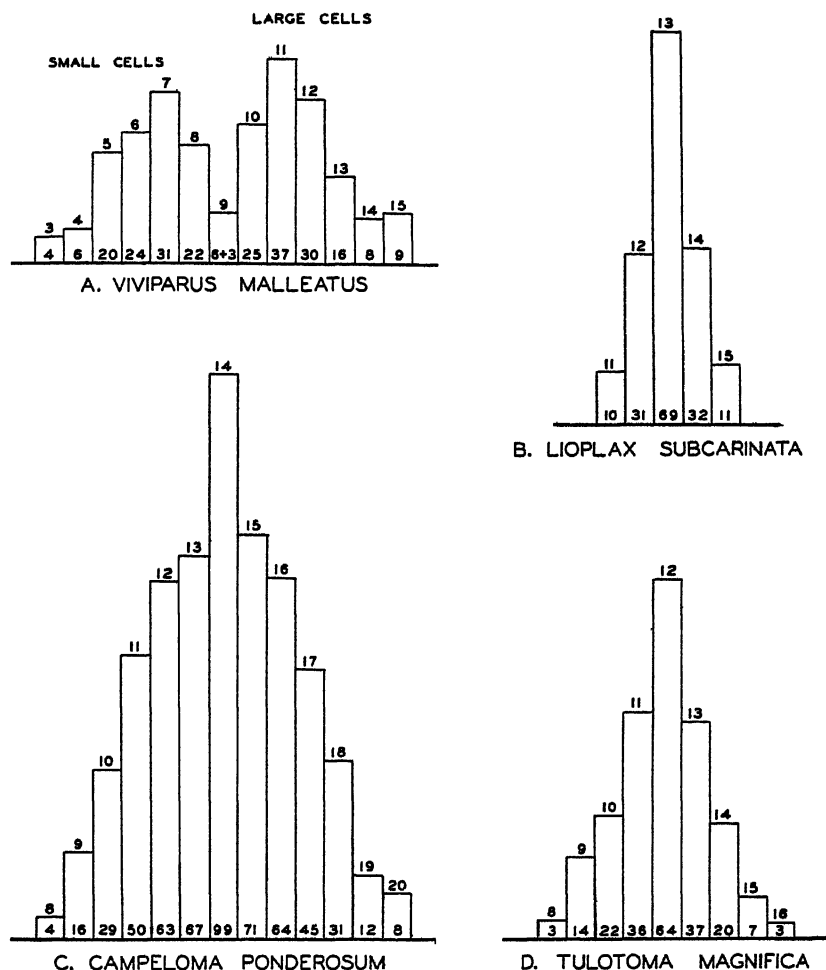
Number of Centrioles

Early in spermiogenesis, the nucleus of the spermatid becomes a lightly staining vesicle, and the chromatin may be destained leaving the centrioles colored. It then becomes easy to make sure counts of the centrioles. These are clearest in stages like FIGURES 72 and 73, where the slight flattening of the nucleus brings all of the proximal centrioles into a single plane (FIGURES 74 and 75). In favorably oriented cells, it is also possible to make sure counts at the earlier stages of FIGURES 69 and 70. In many Hermann-hematoxylin preparations, one may count the axial filaments in cross section. The filament numbers show the same distribution as the centrioles themselves, fully confirming the observation that each centriole gives rise to a single axial filament. In all the charts, except that of *Viviparus malleatus*, counts of both centrioles and axial filaments are lumped together, although, as a check, they are separated in our records.

Meves (1902) stated that in *V. viviparus* there were 12 centrioles at each pole of the second division figure, implying that this number was characteristic of all the cells. In this Meves was wrong. In none of the Viviparidae that we have examined (which includes *V. viviparus*) is the number of centrioles the same in all the spermatids. It varies

over a large (*Campelema*) or small (*Lioplax*) range; and a statistical analysis is necessary to demonstrate the number of centrioles that were present in the spermatocytes, and were allotted to their two descendant spermatids.

Counts of centrioles of spermatids of *Viviparus malleatus* are shown in the population chart of TEXT FIGURE 2A. It will be recalled that



TEXT FIGURES 2. Population charts of number of centrioles in the spermatids in four species of viviparid snails

The number at the top of each column is the number of centrioles; the height of the column and the figure at the base of the column indicate the number of spermatids found to have that number of centrioles. In each case the centrioles were counted in all available cells of several adjacent sections on a single slide.

each second division produced a large and a small spermatid, which can be distinguished from one another at the stage when the centriole counts are made. With the exception of the very small 9-centriole class that is represented by both large and small cells, the larger of the sister spermatids receives more centrioles. Because of this inequality in allotment of centrioles to the two distinguishable types of sister spermatids, it is simple to determine from the data of TEXT FIGURE 2A the number of centrioles that were being thus allotted by the division of each secondary spermatocyte. For, the class represented by both types of spermatid (that with 9 centrioles) must include those cases where each cell receives one-half the total number in the parent secondary spermatocyte; and this number must consequently have been 18 centrioles. This number for the secondary spermatocytes is further confirmed by the fact that, on either side of this common 9-centriole class, the classes that add together as complements to give 18 form approximately the same percentage of the total cells of that type of spermatid counted.* Thus, 19.5 per cent of the larger spermatids had 10 centrioles, 19.5 per cent of the smaller spermatids had the complementary number of 8 centrioles; 28.9 per cent of the larger had 11 centrioles, 27.4 per cent of the smaller, 7; 23.4 per cent of the larger cells had 12 centrioles, 21.2 per cent of the smaller, 6. From these data and from the fact that the two centrospheres of each primary spermatocyte appear to be equal in size, it appears probable that each secondary spermatocyte contains the same number of centrioles, 18.† We may reconstruct the history of the centrioles as follows. Each primary spermatocyte contained two centrospheres, within each of which appeared 9 centrioles (as observed, see p. 16). In the first telophase, each centriole divided so that each secondary spermatocyte received 18 centrioles, which were allotted to the two spermatids in the manner shown by the data of TEXT FIGURE 2A. The total number of centrioles allotted to any four related spermatids, i.e. the descendants of a single primary spermatocyte, was then twice 18, or 36.

Analysis

The main objective of this research was a comparison of centriole and chromosome numbers to determine if the centrioles in excess of the

* Because the presence of the stained mitochondria make it somewhat more difficult to find smaller cells that are favorable for centriole counts, fewer of these cells have been counted.

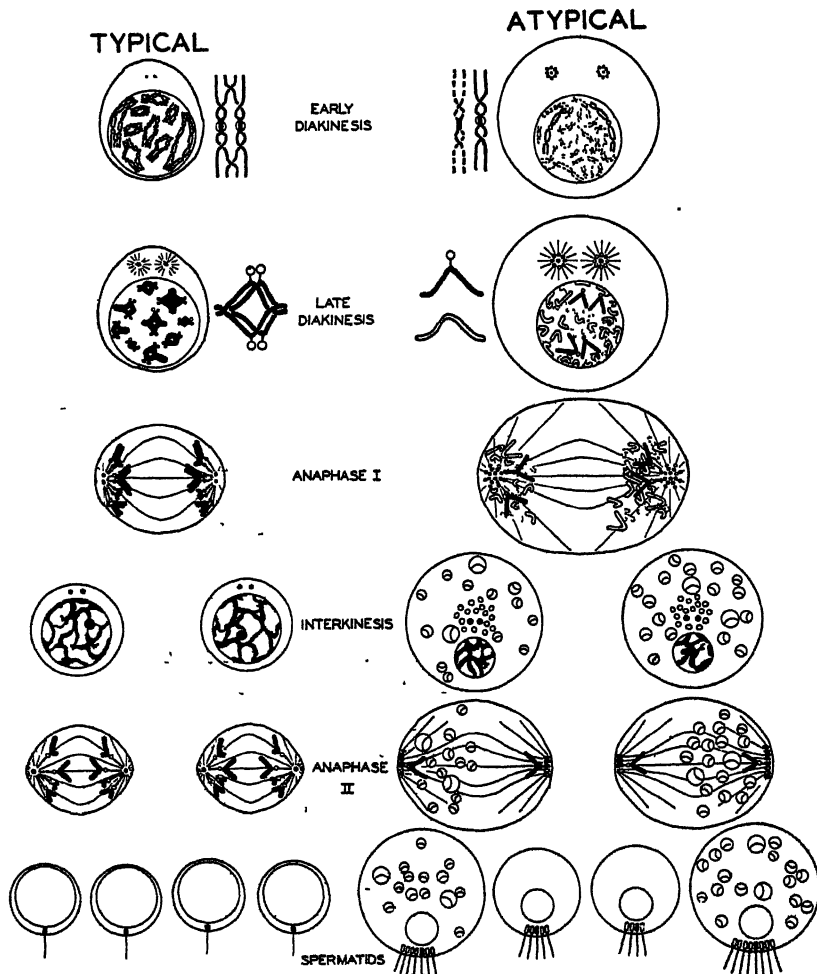
† There is some evidence that the groups of centrioles in each secondary spermatocyte may be subequal. Thus, in two observed cases of failure of division of the secondary spermatocyte in *Campeloma*, the total number of centrioles was 27 and 31 (not exactly 28 in each cell). The conclusions as to the number of centrioles in each quartet of related spermatids is equally valid whether the spermatid centriole numbers are the result of unequal distribution at one division or at two.

normal number could be accounted for on the assumption that each is a centromere that has become detached from its chromosome. Because of the inequality of allotment of centrioles at division II, if one is to deal with a fixed centriole number, he must compare quartets of related spermatids—the descendants of a single primary spermatocyte. The number of centrioles in such a quartet in *Viviparus malleatus* is 36. Since each normal typical spermatid receives a single centriole and each quartet of normal typical spermatids thus has 4 centrioles, the number of extra centrioles to be accounted for in the atypical line is 36 less four, or 32. Turning now to the chromosomes, we have seen above that, of the total of 36 chromatids, only four behaved as if they had centromeres. The other 32 chromatids of each spermatid quartet—those that ultimately degenerated—behaved as if they lacked centromeres, i.e., were acentric. Thus, in each quartet of spermatids from a single primary spermatocyte, the number of missing centromeres, 32, is exactly the same as the number of extra centrioles. For every extra centriole that appears during atypical spermatogenesis, there is one chromosome (chromatid) that behaves as if it were acentric. This coincidence offers the strongest support for the view that extra centrioles in atypical spermatogenesis of *Viviparus malleatus* are not the result of repeated divisions of the original two, as has been formerly believed, but, instead, have arisen by transformation of the centromeres of 32 of the chromatids. Although all the 36 centrioles behave alike they are actually of two sorts: there are 4 *true centrioles*, equivalent to those in a quartet of typical spermatids; and there are 32 of what we may call *centromeric centrioles*, which are in reality detached centromeres.

TEXT FIGURE 3 summarizes in a graphic manner the descriptive and analytical parts of these observations on the double spermatogenesis of *Viviparus malleatus*.

Other Viviparidae

We have also studied three other genera of Viviparidae,—the American forms, *Lioplax*, *Tulotoma*, and *Campeloma*. In the relation between number of acentric chromatids and extra centrioles, these offer complete confirmation of *Viviparus malleatus*. In none of these American forms is the second division unequal. That the other Japanese Viviparidae are like *V. malleatus* is indicated by the observations of Morita (1932) that all of these produce two equal classes of atypical spermatozoa, large and small. In the American genera, the number of centrioles allotted to the two spermatids tends to be more nearly equal



TEXT FIGURE 3. Diagram of our interpretation of the behavior of chromosomes, centromeres, and centrioles in typical and atypical spermatogenesis of *Viviparus malleatus*.

Centromeres (not actually demonstrable in *Viviparus*) are assumed to behave as Schrader has shown for spindle spherules in the spermatogenesis of *Amphiuma*. Centric chromatids are solid black; acentric chromatids are outlined, or are dotted lines; true centrioles, solid black; centromeres and centromeric centrioles are circles.

In the series of typical stages, on the left, each cell has two centrioles and the nucleus contains 9 tetrads. It is assumed that there is a chiasma at either end and that the sister chromatids are relationally coiled as shown in the enlarged tetrads. Each tetrad has at first two centromeres, one to each pair of sister chromatids—a total of 18 centromeres to the whole chromosome complement. By late diakinesis, each centromere has divided, and there are thus 4 to each tetrad, one for each chromatid. At first anaphase, the traction fibers from any two sister centromeres are connected with the same pole. At approximately this stage, each centriole divides—considerably later than the centromeres. At interkinesis, each secondary spermatocyte contains a large nucleus and two centrioles. At the second division, the two sister centromeres have spindle fibers going to opposite poles. Each spermatid receives a full haploid complement of chromosomes and a single centriole—in the group of 4 related spermatids there is a total of 36 chromatids, each with a single centromere, and 4 centrioles.

In the atypical series, on the right, at early diakinesis, the nucleus contains, instead of 9 tetrads, 18 dyads which are pairs of sister chromatids. Two dyads, shown in solid black, are normal and

than in *Viviparus malleatus*, and the degenerating chromatids and the mitochondria are divided approximately equally between the two sister spermatids. Thus there is no cytological method of distinguishing two main types of spermatids as there was in *Viviparus malleatus*. This, however, does not offer any difficulty to the analysis. From comparison with the data of *V. malleatus*, the charts are easily interpreted to show the number of centrioles being distributed by the second division. For, returning to TEXT FIGURE 2A, it is apparent that the 9-centriole class could be distinguished as that resulting from division of the total centriole complement equally between the two daughter spermatids, even if it were not possible, from the cytological differences, to determine that it contained both sister spermatid classes. For, in the chart, the 9-centriole class stands alone. The classes immediately next to it (8 and 10) contain approximately the same number of cells (showing they were produced by a division in which one spermatid received 2 more centrioles than its sister); and the same is true of the 7 and 11 classes and those with 6 and 12. Now in all the charts below (TEXT FIGURES 2B, 2C, and 2D), one spermatid centriole class is similarly flanked by matching classes; and hence this central class must, like the 9-centriole class of *V. malleatus*, represent cases where the centrioles were equally divided between the two daughter spermatids. Just as in *V. malleatus* the recognition of the significance of the 9-centriole class immediately showed the number of centrioles in the secondary spermatocytes to have been 18 (twice 9), so, in all the examples below, the number of centrioles being allotted by the second division can be seen at once by doubling the number in the single central, or unmatched class.

each of these two has one centromere. The other 16 dyads (shown as broken lines) have no centromeres. Presumably, their chromatids are held together by relational coiling. The 16 detached centromeres are outside the nucleus, located in the centrosphere with the two true centrioles. Each centrosphere contains the material of a single true centriole and 8 centromeric centrioles—a total of 18 centrioles to each primary spermatocyte. Next, in late diakinesis, with loss of relational coiling, the sister chromatids separate from one another. The nucleus then contains a total of 36 separate chromatids—4 centric and 32 acentric. The two centromeres that remained on the dyads have now divided, at the normal time for centromere division, so that there is one centromere to each of the four normal chromatids. The detached centromeres, now centromeric centrioles, have not yet divided; nor have the true centrioles. At anaphase of the first division, two centric chromatids move to exact anaphasic positions at each pole; the acentrics move to the general vicinity of the poles. Each of the two true centrioles now divides, at the normal time for centriole division; and also each of the 16 centromeric centrioles divides, though it is considerably later than the time they would have divided had they remained centromeres. Thus, each secondary spermatocyte receives 18 centrioles (two true centrioles plus 16 centromeric centrioles), 2 centric chromatids, and approximately half of the acentric chromatids. At interkinesis, a small nucleus is formed from the two centric chromatids; the acentric chromatids become separate vesicular bodies. At anaphase of the second division, one centric chromatid goes to each pole, and the centrioles collect in two groups—a large group at one pole of the spindle, a smaller one at the other pole. The division produces two unequal spermatids. Each has a normal nucleus, from the single centric chromatid. The larger spermatid has more centrioles and all of the degenerating acentric chromatids. From each centriole, whether true centriole or centromeric centriole, a flagellum grows out. Each quartet of related atypical spermatids has four true centrioles and 82 centromeric centrioles (all shown in the secondary spermatocytes at interkinesis); 82 degenerating acentric chromatids, and 4 normal centric chromatids.

Counts of centrioles in spermatids of *Lioplax subcarinata* are shown in TEXT FIGURE 2B. The single or unmatched class, that of cells with 13 centrioles, must represent cases where the centrioles were divided equally between the two sister spermatids; and each secondary spermatocyte therefore contained 26 centrioles. This is of course also apparent from the fact that the 12-centriole class is approximately equal to the 14-centriole class, and the 11 class equals the 15. Since each secondary spermatocyte had 26 centrioles, there must have been 52 centrioles to each quartet of related spermatids, 48 in excess of the normal 4. The haploid chromosome number in *Lioplax* is 13 (FIGURE 80). Of the total of 52 chromatids, 4 behave centrically and 48 appear to lack centromeres. The presence of 48 extra centrioles is correlated with the presence of exactly 48 acentric chromatids.

In *Campeloma ponderosum* (TEXT FIGURE 2D), 28 centrioles are allotted to each pair of sister spermatids; and each quartet of spermatids that are descended from a single primary spermatocyte has 52 centrioles in excess of the normal 4. The haploid chromosome number is 14 (FIGURE 82). There are 52 acentric chromatids, one for every extra centriole.

In *Tulotoma magnifica* (TEXT FIGURE 2C), each secondary spermatocyte contains 24 centrioles. Hence the number of extra centrioles to each quartet of related spermatids is 44. The haploid chromosome number of *Tulotoma* is 12 (FIGURE 81). Of the total of 48 chromatids, 44 behave as if they had no centromeres. The loss of one centromere from each of these 44 abnormal chromosomes will explain the presence of the extra centrioles.

Summary of Observations and Conclusion

The significant numerical data on chromosomes and centrioles in four species of viviparid snails are summarized in TABLE 1. The haploid or basic chromosome numbers vary from 9 to 14 (column 2). The number of acentric chromatids to each quartet of related spermatids varies from 32 to 52; but, whatever the number of acentric chromatids (column 5), the number of extra centrioles (column 6) always exactly equals it. For every extra centriole that appears during atypical spermatogenesis, there is always a chromosome (chromatid) that behaves as if it had no centromere. Faced with the evidence of such a correlation, the most obvious conclusion is that the extra centrioles appearing in atypical spermatogenesis of Viviparidae are not the result of repeated divisions of the two centrioles normally

found in cells, but, instead, the extra centrioles are centromeres that have left their chromosomes and the nucleus. In the cytoplasm they no longer function as centromeres but behave in all respects like typical centrioles. This enormously reinforces the comparison Schrader has made between centriole and centromeres. For, to the similarity of size, staining reaction, and appearance in living cells we can now add that a centromere placed in the cytoplasm alongside a centriole behaves exactly like a centriole. One can hardly demand stronger evidence for the conclusion that a centriole and a centromere are essentially similar bodies, which normally behave differently because one is isolated in the cytoplasm while the other is in the nucleus as a part of a chromosome.

TABLE 1

Form	N	4N	Centric Chromatids	Acentric Chromatids	Extra Centrioles
<i>Viviparus malleatus</i>	9	36	4	32	32
<i>Lioplax subcarinata</i>	13	52	4	48	48
<i>Tulotoma magnifica</i>	12	48	4	44	44
<i>Campeloma ponderosum</i>	14	56	4	52	52

DISCUSSION

General Considerations

The production of two types of spermatozoa, the typical and the atypical, is characteristic of most prosobranchiate gastropods, the principal exceptions being the more primitive forms, in which only typical spermatozoa occur. The various sorts of atypical spermatogenesis may be arranged in an interesting series (see Ankel, 1930) in which the least atypical spermatozoa are seen in forms like *Fasciolaria* (Hyman, 1923). This atypical spermatozoon differs from the typical ones mainly in being slightly larger and in being unable to activate eggs completely. Increasing atypicity in the series is shown by larger size of the atypical spermatozoa and by the appearance of derangement of the chromosomal mechanism at progressively earlier stages. An extreme type is seen in *Janthina*, which forms an atypical spermatozoon that is a huge cell which is one-third the volume of the egg,

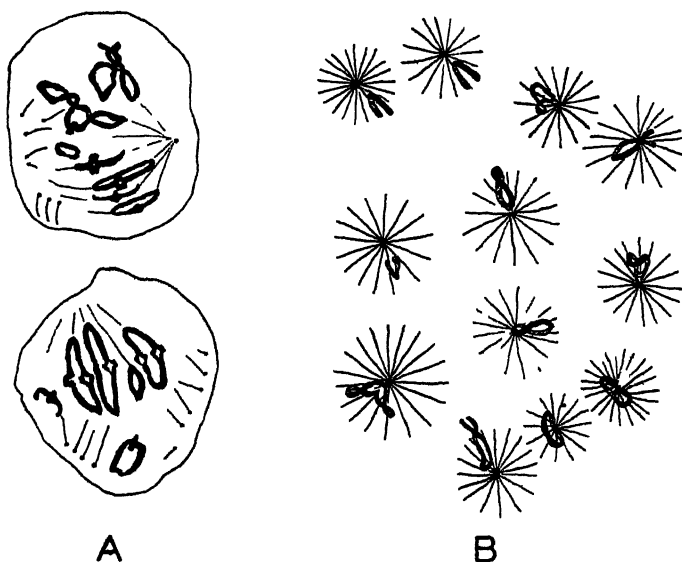
thousands of times the volume of the typical spermatozoon. In all these more extreme varieties of atypical spermatogenesis, the degeneration of all the chromosomes occurs early in the growth period of the spermatocyte—the spermatozoa are thus without nuclear material, or apyrene—and there are, of course, no maturation divisions, the spermatozoon being a transformed primary spermatocyte. In such a series, the Viviparidae obviously fall into a sort of intermediate position, since they have relatively small atypical spermatozoa and only part of the chromosomes degenerate.

Atypical spermatogenesis has been studied in more than forty genera of snails (many are listed in Ankel, 1930). In all but one genus, there is evidence of the presence of extra centrioles in the fact that the atypical spermatozoa are multiflagellate. And in all these cases there is also degeneration of chromosomes. This wide correlation between increase in centriole number and degeneration of chromatin is strong support for the view that the extra centrioles arise, as in Viviparidae, by detachment of the centromeres from chromosomes that are about to degenerate. For this process we suggest the term *decentration*. The atypical spermatogenesis of *Fasciolaria* (Hyman, 1923) is exceptional—but it is the sort of exception that proves a rule. For, in the development of these, the least atypical spermatozoa, no extra centrioles appear; and also there is no degeneration of chromatin.

In most cells, centriolar multiplication is geared to nuclear division, for every cell contains two centrioles that arose by division of the single centriole that was located at the pole of the last previous mitotic figure. Centriole division itself does not get out of gear with nuclear division. The centriole does not divide repeatedly to pile up a large number of centrioles ahead of the nuclear division, although apparently the reverse (endomitosis) can occur.

There have been very few indubitable reports of more than two centrioles to a cell. The atypical spermatocytes of gasteropods have been the classical, and, indeed, very nearly the unique example. This has been universally accepted as demonstrating that centrioles may divide independently of the nucleus. The main point of the present research has been to indicate that this case is really quite different. Another type of cell that contains more than two centrioles is the megakaryocyte of the bone marrow. However, since this is a multinuclear cell, it is by no means certain that the number of centrioles is greater than two per nucleus—or rather two per diploid chromosome complement, for it is quite possible that some nuclear fusion takes place. We have

come across two extremely interesting examples of the apparent detachment of centromeres from chromosomes, outside of atypical spermatogenesis, which are, in some respects, much more striking than the process as noted in the Viviparidae, for they are direct cytological demonstrations and do not involve a statistical analysis. In the progonad of the tadpoles of certain races of bullfrogs, Swingle (1926) has observed the presence of nests of larger spermatocytes in which spermatogenesis is abortive. These cells develop normally up to the metaphase of the first maturation division but no further. The tetrads remain on the metaphase plate and degenerate in that position. As they degenerate—it is not clear whether in its details this degeneration resembles that of the acentric chromatids of Viviparidae—numerous extra centrioles appear in the cytoplasm, and to each of these centrioles a fiber is attached (TEXT FIGURE 4A). These extra centrioles must be the detached centromeres. Indeed, as if for our convenience, each is “labelled” by the fact that its half-spindle fiber is still attached to it. The other example was described by King (1901) in the



TEXT FIGURE 4

A. Redrawn after Swingle, 1926. Abortive primary spermatocytes of bullfrog tadpole. The chromosomes are degenerating in the metaphase position. The numerous centrioles with attached fibers may, in reality, be detached centromeres, with their half-spindle fibers.

B. Redrawn after King, 1901. Chromosomes at prometaphase of the first maturation division of the toad. The aster near each chromosome may indicate that the centromeres have left their chromosomes and assumed the characteristic centriolar property of organizing an aster.

oocytes of the toad. She noted a late prophase of the first maturation division, after the disappearance of the nuclear membrane, in which, close to each chromosome, there was a small aster (TEXT FIGURE 4B). King described this as a part of the normal maturation process, but no transitional cells connecting it with earlier or later stages were noted. We suggest that this was really an abnormal oocyte in which decantation had just occurred, and the centromeres were caught at the very moment of leaving their chromosomes, becoming centrioles, and organizing asters.

In Viviparidae, as we have seen, the separation of the centromere material from the chromosomes probably takes place in a period that corresponds to a stage of typical spermatogenesis between early leptotene and early diakinesis; for the centrosphere does not grow after this latter time, and the full number of centrioles is demonstrably present before the end of diakinesis. However, the transformation of centromeres into centrioles is not, in snails as a group, restricted to this period. For instance, Kuschakewitsch (1913) has shown that in *Vermetus* the extra centriolar material does not appear until just before the breakdown of the nuclear membrane, i.e., the end of diakinesis; and in *Cerithium* (Kuschakewitsch, 1921), the extra centrioles first appear during the course of the first maturation division. There does appear to be one limitation to the time when transformation of centromeres into centrioles can take place: there is no case of appearance of extra centrioles after the beginning of the development of the flagellum.*

The Centrioles

Space does not here permit extended discussion of the problem of the status of the centriole. For this the reader is referred to the admirable ninth chapter of Wilson's "The Cell in Development and Heredity." In brief summary, there are grounds for the fairly general agreement on certain main facts about the morphology and physiology of centrioles. First, each animal cell typically contains two centrioles, in the form of small granules, considerably less than one-half micron in diameter, which stain characteristically with certain basic dyes, not-

* Many Lepidoptera form apyrene spermatozoa, and the chromatin degeneration process seems to be like that of the acentric chromatids of atypical gastropod spermatogenesis (see e.g., Meves, 1902). In no instance, however, are the spermatozoa multiflagellate, nor are there extra centrioles. This may be correlated with the fact that, in the normal spermatogenesis of Lepidoptera, the development of the flagellum is precocious; and there are four, one for each spermatid, already present in the growth period of the spermatocytes. Thus, in Lepidoptera, throughout the time when, judging from what occurs in snails, transformation of centromeres into centrioles might be expected to take place, it seems that the cytoplasmic conditions are like those of the early spermatids of snails—conditions that appear to be unfavorable for the centromere-centriole transformation.

ably hematoxylin and crystal violet. In interphase, the two centrioles are closely adjacent to one another, and the pair are in a characteristic location in each type of cell, e.g., in the distal end of a columnar epithelial cell, and near the center of the main cytoplasmic mass in a leucocyte (see Pollister, 1933). The two centrioles move apart at mitosis and take positions that later come to be the poles of the spindle, and each centriole organizes the immediately adjacent cytoplasm into an aster. The one certain function of the centriole is to act as a blepharoplast. In unicellular organisms and in sperm-forming cells and some simple epithelia of higher animals—wherever flagella and their associated elements appear—the centriole is intimately concerned in their formation. In the simplest cases, like unflagellated epithelial cells (Pollister, 1933), the flagellum grows out directly from that one of the two centrioles that is nearer the free or lumen end of the cell. Since the flagellum-forming centriole remains the same size as its fellow that has not formed a flagellum, it seems clear that the flagellum is not an outgrowth or elongation of the centriole substance itself. Instead, the blepharoplast function must involve first a localization of flagellum substance in the vicinity of the centriole—either by causing its accumulation from the surrounding cytoplasm, or by promoting its synthesis from substrates—and this must be followed by a second step of spinning out the substance into flagellum. In these simplest cases, there is no visible piling up of flagellum material about the centriole. It must become flagellum almost as rapidly as it reaches the centriole. In unicellular flagellated organisms and in spermatids, however, it is a common condition that a mass of material accumulates around the centriole so that the centriole and associated material form a body much larger than any true centriole—sometimes, in fact, it is measurable in microns (see Cleveland, 1934). In some instances, this substance becomes spun out into flagellar structures directly from its juxtapacentriolar position, and, when all of it has thus been used up, the centriole appears in its original size (e.g., *Murgantia*, Bowen, 1922). More commonly, however, the flagellum-forming substance separates from the true centriole and becomes an apparently quite distinct entity—the distal centriole or centrioles of spermatids or the blepharoplast of Protozoa (see Swezey, 1916)—and these structures may become differentiated into a bewildering variety of flagella and accessory flagellar structures (see Cleveland, 1934). The confusion of this flagellum-forming material with the true centriolar substance, which latter is alike in both flagellated and nonflagellated cells, has darkly

clouded some aspects of the problem of centriole reproduction. Hence, it is well to keep in mind that flagellum material is quite distinct from the flagellum-inducing organoid, the true centriole. It appears to us that there are good criteria which, in a complete analysis of any situation, will serve to differentiate unmistakably between these two: the flagellar substance is sporadic and transitory, appearing only in those cells that are about to form flagella; the total volume can only increase as new flagellar material is formed about the true centriole; although a mass of flagellar substance can become divided up into a number of separate, and often functionally quite different elements, this division is related to these functional demands and hence is not related to the cycle of cell division. The true centriolar substance, by contrast, is present in the same amount in all cells of an organism, and its increase and division are then necessarily closely geared to the division cycle of the cell, especially of the nucleus. It is this last aspect of the centriole problem—the manner of maintenance of the normal complement of two centrioles to a cell—that has aroused most controversy, and we now turn to a discussion of this question in the light of the results reported in the present paper.

There has been much dispute about the mode of growth and reproductive potentiality of centrioles. On the one hand, it is abundantly clear in many cases that the two centrioles of the interphase cell have arisen by division of the single one at the pole of the last previous mitotic division figure; and it has been inferred, therefore, that the centriole is a self-perpetuating body in the same sense as some part of a chromosome must be. On the other hand, there is considerable evidence that, unlike the chromosome, a centriole can arise in some manner other than by division of a pre-existing centriole. For there are instances—notably the spermatogenous cell generations in ferns—where typical centrioles seem to arise in cells that for many previous cell generations lacked centrioles. In the face of this sort of evidence, it is impossible to maintain the strict view that centrioles, like chromosomes, arise only by self-duplication of pre-existing individualized centrioles, in a manner analogous to chromosome reproduction. Nevertheless, the constancy of size and of number of centrioles in animal tissue cells, which are themselves very diversely specialized, argue powerfully against the centriole being a temporary cell organoid that comes and goes in response to functional demands. How can these apparently contradictory phenomena be reconciled, and how can a consistent view of the nature of the reproduction of centriolar material be

developed? The answer seems to us to be indicated by two lines of evidence: first, the time of growth and division of the centriole; and, second, the demonstration that the centromere (the genetic continuity or constancy of which is assured since it is produced by the chromosome) is the same substance as the centriole, and hence is a constant potential reservoir of centriole substance. In brief, we suggest that the normal method of centriole growth is by contribution from the centromere, usually through a flow of material along the half-spindle component, which connects centromere directly with centriole.

Although their small size practically precludes accurate measurement of growth, it is evident in many figures in the literature that the centriole is largest when at a spindle pole, and that therefore its growth occurs during the time when it is in that position. It is usually most conspicuous at metaphase or early anaphase. With the exception discussed below (page 33), the division of the centriole takes place only after it has been in direct connection with the centromere through the half-spindle component, i.e., not before metaphase (TABLE 2). The sequence of significant events in most typical cell divisions is as follows: the centriole takes its position at a spindle pole; the nuclear membrane breaks down; the chromosomes assume the equatorial plate position so that centromeres and centriole are connected by the half-spindle component; the centriole grows; the centriole divides. It appears to us to be a logical interpretation of this sequence that the division of the centriole occurs at this particular time because it grows as a result of a small addition of material from each centromere, the total contributions from all the centromeres being enough to double the size of the centriole and thus provide for its later division into two centrioles of the standard interkinetic size. There is further support for this view of the source of centriolar material in the fact that frequently it has been observed that the half-spindle components stain quite unlike the other fibrous elements of the spindle and like the centrioles and centromeres. This is especially true of cells stained by the Benda method. This may well be an actual demonstration of centromere substance flowing along the half-spindle to be added to the centriole.

The concept of centriole reproduction we have outlined above occupies a sort of middle ground between the two opposing views of strict genetic continuity and occasional "*de novo*" origin. It states that no centriole is in fact capable of independent self-duplication, in the chromosomal sense of synthesizing its own substance from substrates in the surrounding cytoplasm; that centrioles are, however, constant fea-

TABLE 2

Form and Cell Type	Time of Centriole Division	Reference
Somatic Cells		
Trout. Blastodisc	anaphase	Henneguy, 1891
Salamander. Gill epithelium.	anaphase	von Erlanger, 1896
<i>Chaetopterus</i> . Blastomeres	metaphase	Mead, 1898
<i>Salpa</i> . Epithelium of pharynx	telophase	Ballowitz, 1898
<i>Cerebratulus</i> . Blastomeres	anaphase	Coe, 1899
<i>Thalassema</i> . Blastomeres	metaphase	Griffin, 1899
<i>Ascaris</i> . Blastomeres	metaphase	Boveri, 1901
<i>Echinus</i> . Blastomeres	metaphase	"
Duck. Erythrocyte	anaphase	Heidenhain, 1907
<i>Lumbricus</i> . Amoebocytes	anaphase	Joseph, 1910
Goblet-cells	anaphase	Tschassownikow, 1914
Amphibia. Leucocytes	telophase	Belar, 1926
Dogfish, Acinar cells of pancreas	metaphase	Pollister, 1933
<i>Oecanthus</i> . Spermatogonia	anaphase	Pollister, 1929
<i>Drosophila</i> . Blastema	anaphase	Johnson, 1931
		Huettnr 1933
Oocytes		
<i>Asterias</i> . Polar division I.	metaphase	Wilson and Matthews, 1895
<i>Diadema</i> . I.	anaphase	MacFarland, 1897
<i>Arenicola</i> . I.	metaphase	Child, 1897
<i>Chaetopterus</i> . I.	metaphase	Mead, 1898
<i>Zirphaea</i> . I and II.	anaphase	Griffin, 1899
<i>Cerebratulus</i> . I.	metaphase	Coe, 1899
<i>Thalassema</i> . I.	anaphase	Griffin, 1899
<i>Uro</i> . I.	anaphase	Lillie, 1901
Spermatocytes, Division I		
<i>Salamandra</i> .	anaphase	Meves, 1897
<i>Ascaris</i>	metaphase	Sturdivant, 1934
<i>Euschistus</i>	pachytene?	Montgomery, 1911
<i>Gelastocoris</i>	pachytene?	Payne, 1927
<i>Gerris</i>	diakinesis	Pollister, 1930
<i>Opisthocanthus</i>	pachytene?	Wilson, 1931
<i>Oecanthus</i>	pachytene?	Johnson, 1931
<i>Anisobatis</i>	diakinesis	Schrader, 1941

tures of cells, because at each cell division the volume of centriolar substance is doubled by addition of its specific material from the centromeres; that this contribution of centriolar substance will occur at every division, because centromeric material (which is identical with that of the centriole) is synthesized by the constant self-reproducing cellular units, the chromosomes. In its broader aspect, the centriole is a bit of specific chromosomal product that functions in the cytoplasm. Hence, wholly aside from its special significance, e.g. as an inducer of the flagel-

lar structures characteristic of the particular organism in which it occurs, centriole reproduction is worthy of high consideration as a possible model of the method of influence of the nucleus on the cytoplasm. For here we have the nucleus producing a special substance which passes out into the cytoplasm; there it functions as a concentrator or synthesizer of materials for special cytoplasmic differentiations, the flagellar elements.

It is illuminating to re-examine the diverse phenomena of centriole reproduction in the light of the view that the sole site of synthesis of centriolar material is the centromere-producing part of the chromosome. As we have already seen, in most cases, this intrachromosomally synthesized centromere-centriolar substance is added to the centriole at metaphase-anaphase when it flows out along the half-spindle components. Conspicuous exceptions to this are seen in certain prophases of the first male meiotic division (TABLE 2) where, since centriole growth and reproduction unquestionably occur before breakdown of the nuclear membrane, it must be assumed that the centriolar substance diffuses through the nuclear membrane, probably at its idiosomal pole. Parenthetically, it is to be noted that the phenomena in prophase of the atypical meiosis of Viviparidae appear as merely an extreme exaggeration of this process of passage of centromere-centriole substance through the nuclear membrane. If centromere material can be added to the centriole at any time within a considerable period of mitosis (that is from early prophase as in some spermatocytes to early anaphase) and if new centrioles can arise by transformation of centromeres, then the remaining evidence that the centriole possesses a capacity for self-duplication is indeed slight. There appear to be but two possible lines along which conclusive evidence of this sort of reproduction could be obtained. First, by genetical proof analogous to the demonstration of plastid inheritance—a line that has apparently never been explored to the slightest extent. The cytological proof, the second line, could only come from a conclusive demonstration that centrioles can continue to multiply in the complete absence of nuclear material. The observation of progressive increase in number of cytasters in anuclear, parthenogenetic merogenes (Harvey, 1936) is typical of a considerable body of evidence that has been interpreted as demonstrating that centrioles are capable of self-reproduction. This appears to us by no means conclusive. The presence of a cytaster does not necessarily imply the existence of a centriole within it. The astral configuration is primarily to be regarded as a consequence of stresses (e.g.,

flow or mechanical) which are radial to a point. Although the centriole appears at times to cause such radial stresses and to bring about the special molecular arrangements that we can detect as astral rays, there are conspicuous and perfect asters which do not center in the centriole but are, instead, a consequence of radial flow due to the relation of the cell to the surrounding fluid, e.g., the aster of a leucocyte (Pollister, 1933 and 1941). The possibility must be considered that the special conditions of artificial parthenogenesis may cause the formation of numerous noncentriolar asters, the cytasters. In short, the appearance of cytasters in parthenogenetic merogones and the later increase in number of cytasters are not-conclusive evidence that centrioles may appear *de novo*, or are capable of self-duplication. Although these cytasters are undoubtedly of great interest in connection with the mechanics of aster formation, they may well have no significance whatever to the problem of centriole reproduction.

The view that the approximately final source of centriolar material is the nucleus—more particularly the centromeric regions of the chromosomes—finds strong support in the otherwise perplexing observations of the appearance of centrioles in the spermatogenous divisions of certain plants, from the vegetative divisions of which centrioles appear to be lacking. For example, in *Marsilia*, Sharp (1914) noted that the centrioles first appear in early anaphase. The spindle at metaphase is the blunt one characteristic of the vegetative divisions. In early anaphase its fibers become reorganized so that they focus at a point. At this point a centriole is detectable, and about this centriole an aster forms. It is highly significant, that the moment of appearance of this plant centriole is immediately after the metaphase, the stage when in animal somatic cells the centriole grows, because, we believe, it receives centriolar material from the centromere. The appearance of centrioles in these spermatogenous divisions of plants has always been most difficult to reconcile with the doctrine that centrioles arise only by division of pre-existing centrioles. There is little difficulty if it be recognized that the centromeric regions of the chromosomes are sites of synthesis of centriolar material, and hence potentially capable of contributing to the formation of a cytoplasmic centriolar body at any time. Indeed, the moment of appearance of this plant centriole is exactly that when it would be predicted from analogy with the time of growth of the centriole in somatic mitosis of animal cells. The centriole of spermatogenous cells of *Marilia*, *Equisetum*, etc., is thus very readily interpretable as revival, under the special functional demands

of cells that are about to become flagellated spermatozoa, of a capacity of the centromeres to produce centriolar bodies by flow of material along the half spindle components—a capacity dormant in all vegetative divisions.

There is support for the notion that the centriole is primarily a nuclear body in observations on centrioles in Protista (see especially Belar, 1926). In many of these forms the mitosis is entirely intranuclear, and the centriole appears never to escape from the nucleus to become a cytoplasmic organoid. This presumably represents the primitive condition, from which the condition in higher animals is but the same step as that by which an intranuclear mitotic figure becomes extranuclear. That this step has actually never taken place in the higher plants is a theory that offers extremely interesting possibilities for explaining the centriole situation in such plants.

The Chromosomes

According to our interpretation of the data on Viviparidae, in the nucleus of the atypical primary spermatocyte, from approximately diplotene onward, all but a few of the chromatids lack centromeres; otherwise they are complete chromosomes. Although there have been observations of acentric chromosomal fragments (e.g., Carlson, 1938), these chromosomes of *Viviparus* seem to be the first examples reported of chromosomes that are entire except for the centric mechanism. We are thus provided with an opportunity to infer something of the physical relations between chromosome and centromere; and, from the behavior of the incomplete or acentric chromosomes, to determine which of the properties of complete chromosomes are referable to the centric region and which to the rest of the chromosome.

Some theoretical interest attaches to the fact that the acentric chromatids are little altered by the removal of the centromere. It has been suggested that the centromeric region is best pictured as a bead on a string (Darlington, 1939). From this presumed intercalated position it would be expected that the centromere could be removed only by breaking the string, and thus separating the two arms of the chromosome. Indeed, while our preliminary report on *Viviparus malleatus* was in press, Darlington's paper (1939) on misdivision appeared—containing the first suggestion of the possibility that centromeres becoming completely separated from the chromosomes might become "disembodied centromeres." Darlington found that the isola-

tion of the centromere by misdivision did, in fact, separate the two arms of the chromosome, as theory predicted, thus presumably breaking the "fibrous connection" running through the centromere and uniting the two arms. In the decentration process in *Viviparus*, however, the two arms of the acentric chromosome remain together; and the most likely explanation of this is that the centric material has been removed, leaving the fibrous connection between the arms intact. Continuing the string of beads analogy: one may assume as a model that the genes are glass beads while the centromere is a bead of wax. At low temperatures, the wax bead could only be isolated by cutting the string on either side of it (misdivision); but, at high temperatures the wax bead would be melted off, leaving the string unbroken (decentration).

From the data in Viviparidae, it appears that two items of behavior of chromosomes are due to properties of the chromatin and not dependent upon the presence of the centromere. First, the acentric chromatids show the typical diakinetic orientation, which involves two events: the movement to the nuclear periphery and the arrangement in equal spacing thereon. Second, whether centric or acentric, the chromosomes show a clear tendency to go to one of the poles of the first division soon after the nuclear membrane breaks down. Carlson (1938) has made a careful study of the behavior of acentric fragments in somatic mitoses of the neuroblasts of the grasshopper. He reports results somewhat different from ours. His fragments, at metaphase, assume an equatorial position outside the spindle; and there they remain until late anaphase when, if they come into relation with the spindle, they may divide and belatedly move to join the centric chromosomes at the poles. These acentric fragments are not, in every way, strictly comparable with the acentric chromatids of Viviparidae; and in these differences may lie the possibility of explaining the discrepancies in behavior. Carlson was dealing with *two* sister fragments of somatic chromosomes. We are concerned with *single* whole meiotic chromatids. For the equatorial orientation at metaphase, it is quite conceivable that one must have either two associated sister chromosomal elements or two synapsed homologues, and neither of these two pairings occurs at the first maturation division of Viviparidae. Carlson's fragments are perhaps nearly normal, for they do not degenerate for some time. The chromatids of Viviparidae, however, are very close to becoming abnormal, since they cannot go into normal interphase condition at telophase I. While there are thus differences between Carlson's results

and ours, we wish to emphasize strongly that both agree in showing that chromosomal material can move to the pole in the complete absence of attached centromeric material. This appears to signify either that the centromere does not have quite the central role in anaphase movement that it has often been assumed to have, or else that there is a sort of double assurance that chromatin is to reach a spindle pole. We wish, furthermore, to emphasize that our results lead us to conclusions in complete accord with Carlson's opinion that ". . . the functions of the kinetochores are primarily to make uniform the orientation of the chromosomes in the equatorial plane at metaphase and to synchronize the middle and late anaphase separation of daughter chromosomes in order to insure their equal apportionment to the daughter cells."

SUMMARY

The centrioles and chromosomes have been intensively studied in typical and atypical spermatogenesis of *Viviparus malleatus*, and brief confirmatory observations have been made on three other species of viviparid snails.

Typical spermatogenesis has no unusual features. The two nucleoli fuse when their associated chromosomes synapse. This fusion nucleolus disappears before the stage of diakinesis. The two centrioles of the first meiotic prophase take positions at the idiozomal pole of the nucleus in leptotene and they remain there until they move apart to the spindle poles of the first division. The centrioles divide in the telophase of the first division, and one of the four thus formed is included in each of the four spermatids.

Atypical spermatocytes become much larger, due to more cytoplasmic growth. There is no synapsis. At a stage which seems, from the disappearance of the nucleolus, to be early diakinesis, the sister chromatids separate from one another. As a consequence, in late diakinesis the nucleus contains 36 (4N) separate chromatids instead of 9 (N) tetrads. Of these 36 chromatids, 4 show approximately normal anaphasic behavior at the two meiotic divisions. The other 32 behave as if the mitotic orienting mechanism (the centromere, kinetochore, or spindle attachment point) were nonfunctional or absent. The 32 abnormal chromosomes degenerate, disappearing in the early spermatids.

In atypical spermatocytes, the two centrioles grow to be large spheres of equal size, the centrospheres, during the spermatocyte growth period. There is evidence that the growth of the centrosphere is a result of the accretion of material that flows out of the nucleus at its

idiozomal pole. At late diakinesis, each centrosphere becomes resolved into a number of centrioles, which, in size and staining character, are like the two of normal spermatocytes of the typical line. Each of these centrioles divides in telophase I, at the normal time for centriole division. At prophase of the second division, each centriole is surrounded by a small aster; and each centriole later goes to one pole of the division figure. In most cases in *Viviparus malleatus*, one spermatid receives a larger number of centrioles. The atypical spermatozoon is multiflagellate because each centriole functions as a blepharoplast.

From a statistical study of the number of centrioles in the spermatids, it is evident that each quartet of related spermatids (those descended from a single primary spermatocyte) contains 36 centrioles—an excess of 32 over the normal 4 centrioles. This number of extra centrioles agrees exactly with the number of chromosomes that behaved as if they lacked centromeres—as if they were, in Darlington's terminology, *acentric*. From this numerical comparison, it is concluded that the 32 extra centrioles are actually the transformed centromeres of the 32 acentric chromatids; that of the total 36 centrioles there are 4 true centrioles (corresponding to the 4 of the typical line) and 32 centromeric centrioles. The centriole and chromosome counts in representatives of three other genera of viviparid snails, which have different chromosome numbers, likewise show that the number of extra centrioles always agrees exactly with the number of chromatids that behave as if acentric.

From the fact that all the centrioles of the atypical line behave like normal centrioles, and show no individual differences in function, it is concluded that centromere and centriole are essentially similar bodies that normally show differences in behavior, only because one is in the nucleus as a part of a chromosome while the other is in the cytoplasm.

Thus it is held that the extra centrioles in atypical spermatogenesis of viviparid snails do not arise, as has always been supposed, by repeated divisions of the two centrioles. Instead, the increased number of centrioles is a result of the contribution from the nucleus, in the form of centromeric substance. In the discussion it is pointed out that, in a normal centriole cycle, the centriole divides only after it has been in close connection with the centromere by the half-spindle fiber; and it is suggested that the division of the centriole is normally a consequence of its receiving an addition of centromeric substance flowing along the half spindle. The general view that centriolar material always originates from the nucleus is developed, and it is pointed out

how this explains some of the hitherto puzzling centriolar phenomena—for example, the appearance of centrioles in the spermatogenous divisions of plants that lacked centrioles in the vegetative divisions.

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EXPLANATION OF PLATES

All figures are camera lucida drawings. The magnification, as reduced, is 1300 times. All figures, except 49-51 and 80-82, are from *Viviparus malleatus*. The method of fixation and staining are given after each figure description.

PLATE 1

All figures are from the typical line of *Viviparus malleatus*.

FIGURE 1. Spermatogonium, showing two nucleoli. Benda's fixation, alizarin-crystal violet stain.

FIGURE 2. Spermatogonium, showing two centrioles on periphery of the idiozomal area. Hermann's fixation; iron hematoxylin staining.

FIGURES 3, 4. Spermatogonial prophase, upper surface of nucleus (3) and middle focal plane of nucleus (4). These spermatogonial chromosomes are on the nuclear periphery as in a typical meiotic diakinesis. Benda; alizarin-crystal violet.

FIGURE 5. Spermatogonial metaphase. 18 V-shaped chromosomes. Hermann, hematoxylin.

FIGURE 6. Spermatocyte, early growth stage, probably preleptotene. Two small nucleoli. Hermann, hematoxylin.

FIGURE 7. Spermatocyte, leptotene. Nucleoli (only one of two is shown) are much larger. Benda, alizarin-crystal violet.

FIGURE 8. Spermatocyte, leptotene. Centrioles close together and near the idiozomal pole, where they remain until late diakinesis. Hermann, hematoxylin.

FIGURE 9. Spermatocyte, pachytene, showing chromosomes with ends in contact with idiozomal side of nuclear membrane. Note that the two nucleoli have fused. The single fusion nucleolus at this stage is always associated with a region near the end of one of the longer chromosomes. Benda, alizarin-crystal violet.

FIGURE 10. Pachytene chromosomes seen from the idiozomal pole, showing the 18 ends of the 9 looped chromosomes. The entire length of two short chromosomes is shown. Flemming, Newton's gentian violet.

FIGURE 11. Lower focal plane of same nucleus as FIGURE 10. Note that four chromosomes are evenly spaced on the nuclear periphery, those of intermediate length are seen here as loops extending across the center of the nucleus. Note unsynapsed region in central chromosome. From FIGURES 9, 10, 11, it is evident that, at pachytene, the chromosomes are evenly spaced on the periphery of the nucleus, so far as they can be without detaching their ends from the idiozomal pole of the nucleus. Hermann, gentian violet.

FIGURE 12. Nucleolus-bearing chromosome as it is synapsing. Benda, alizarin-crystal violet.

FIGURE 13. Early diakinesis; 9 tetrads. Hermann, hematoxylin.

FIGURE 14. Late diakinesis; 9 tetrads. Hermann, hematoxylin.

FIGURE 15. Late diakinesis. The two centrioles are moving apart along the nuclear membrane and a small aster has developed about each of them. Hermann, hematoxylin.

FIGURE 16. First prophase. Nuclear membrane has broken down and the tetrads, hitherto widely spaced, have drawn together into a clump. Hermann, hematoxylin.

FIGURES 17, 18. Metaphase, first division, 9 tetrads. Hermann, hematoxylin.

FIGURE 19. Telophase, first division. Centrioles have just divided as seen at left pole. Hermann, hematoxylin.

FIGURE 20. Interkinesis. The two centrioles are near the center of the main mass of cytoplasm. Hermann, hematoxylin.

FIGURE 21. Prophase of second division. Centrioles have taken positions on the nuclear membrane as at leptotene of division I. Chromosomes are evenly spaced on the periphery of the nucleus. Benda, alizarin-crystal violet.

FIGURE 22. Prophase of second division. Chromosomes have clumped following breakdown of nuclear membrane. Benda, alizarin-crystal violet.

FIGURE 23. Metaphase of second division. Centrioles at spindle ends on periphery of the cell. Hermann, hematoxylin.

FIGURE 24. Anaphase of second division. Each centriole slightly elongated. Hermann, hematoxylin.

FIGURE 25. Telophase of second division. Chromosomes on nuclear periphery. Flagellum has developed from each centriole. Hermann, hematoxylin.

FIGURE 26. Spermatid at chromatin shell stage. Centriole divided into proximal and distal parts. Hermann, hematoxylin.



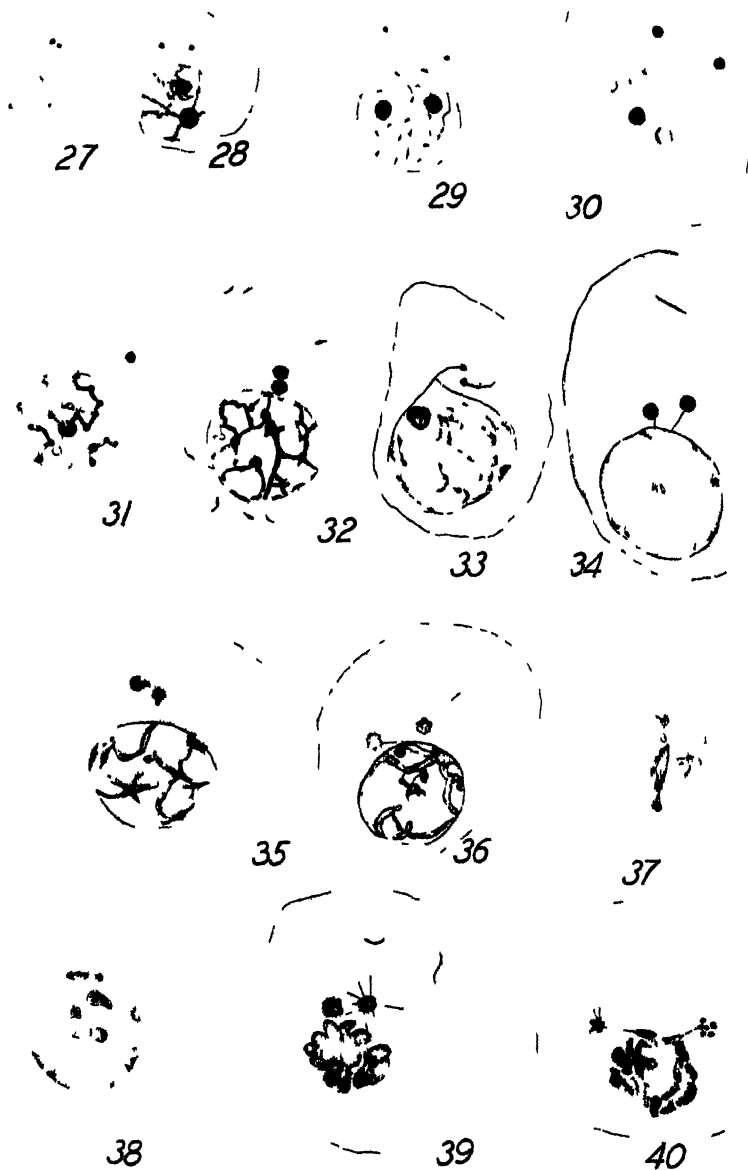


PLATE 2

All figures are atypical primary spermatocytes of *Viviparus malleatus*.

FIGURE 27. Earliest stage at which atypical spermatocytes can be distinguished. Two centrioles on periphery of idiozomal region. Benda, alizarin-crystal violet.

FIGURE 28. Early growth stage. Two nucleoli at maximum size. Benda, alizarin-crystal violet.

FIGURE 29. Probably leptotene, nucleoli somewhat smaller. Centrioles still on periphery of much enlarged idiozome. Benda, hematoxylin.

FIGURE 30. Later growth stage, beginning of growth of the centrospheres. Benda, alizarin-crystal violet.

FIGURE 31. Like FIGURE 30, but better fixation of chromosomes. Benda, hematoxylin.

FIGURE 32. Stage of maximum size of centrospheres. Nucleoli have disappeared. Chromosomes now on nuclear periphery. Benda, alizarin-crystal violet.

FIGURE 33. Intermediate stage of centrosphere growth. From a nuclear lobe a filament extends to one centrosphere, other centrosphere has filament from surface of idiozome. Hermann, hematoxylin.

FIGURE 34. Later stage in centrosphere growth. Each sphere is connected with nucleus by a stainable filament. Benda, alizarin-crystal violet.

FIGURE 35. Stage when each centrosphere has small lobe approximately the size of the later centrioles, e. g., in FIGURE 40. Benda, alizarin-crystal violet.

FIGURE 36. Early diakinesis, chromosomes peripheral, centrospheres moving apart and becoming mulberry-like; an aster has developed about each centrosphere. Benda, alizarin-crystal violet.

FIGURE 37. Middle diakinesis. Sister chromatids partially separated. Hermann, gentian violet.

FIGURE 38. Late diakinesis. Chromosomes, approximately 36 in number, are evenly distributed on the periphery of the nucleus. Hermann, gentian violet.

FIGURE 39. Immediately after breakdown of the nuclear membrane. Chromosomes in compact group. Benda, alizarin-crystal violet.

FIGURE 40. Spindle developed. Well-developed half spindles. Hermann, hematoxylin.

PLATE 3

All figures are atypical spermatocytes in the first maturation division.

FIGURE 41. Showing 9 centrioles in the group at left pole. More commonly the centrioles are clumped as at the right pole and it is then not possible to determine their exact number. Note half spindle to left pole. Benda, alizarin-crystal violet.

FIGURE 42. Two chromosomes in a possible metaphase position. Such appearances are common enough to suggest that the centric chromatids may attempt to take up a metaphase orientation. That perfect metaphase orientation of these normal chromosomes is not observed is probably due to the fact that the movement of the normals is hampered by the presence of the much more numerous acentric chromatids. Hermann, hematoxylin.

FIGURE 43. Polar view of anaphase, showing random orientation of the V-shaped chromosomes. At this stage, centric chromatids should have the apices of the V's pointed directly toward the group of centrioles that marks the spindle pole. Hermann, hematoxylin.

FIGURE 44. Anaphase. Chromosomes poorly fixed. Benda, alizarin-crystal violet.

FIGURE 45. Anaphase, entire cell from thick section. 36 chromatids, of which all but 8 are at the spindle poles. Chromosomes not well fixed. Hermann, gentian violet.

FIGURE 46. Early anaphase. Exceptionally well fixed. Hermann, hematoxylin.

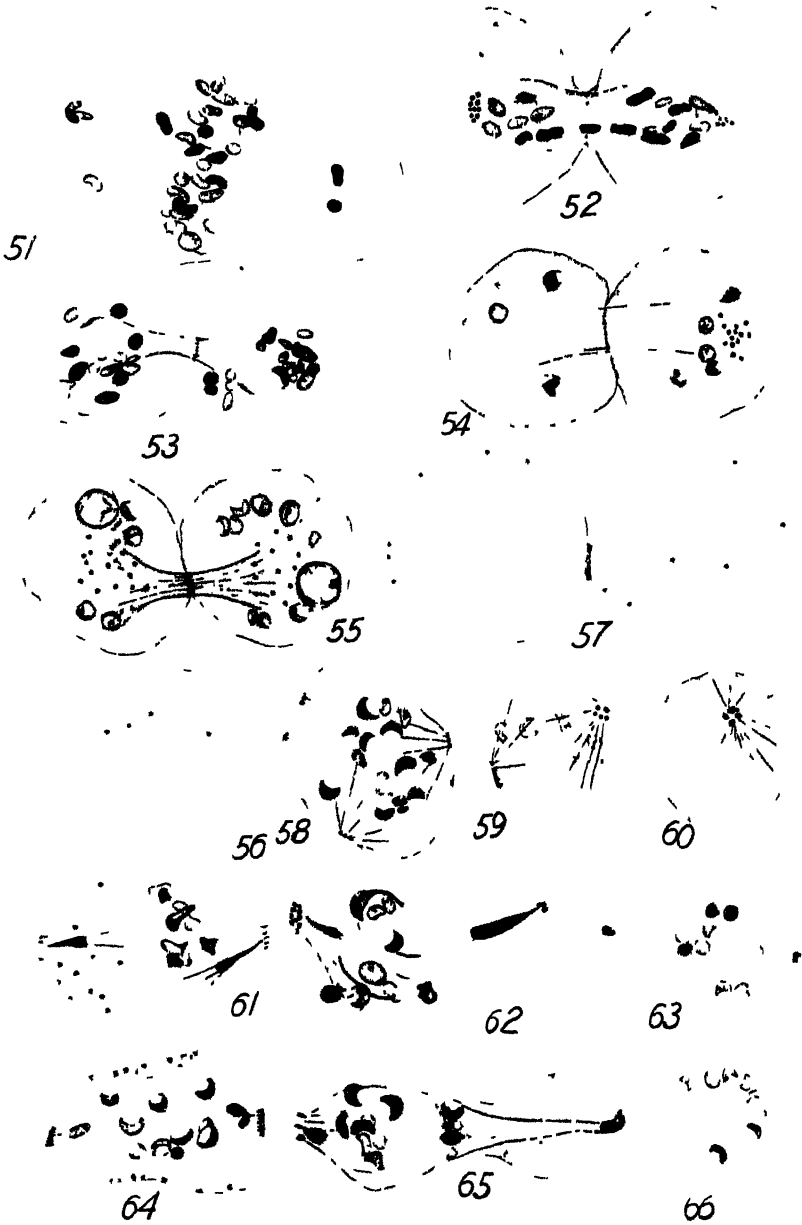
FIGURE 47. Anaphase, from a smear preparation. Full complement of 36 chromatids. Hermann, gentian violet.

FIGURE 48. Late anaphase, after many chromosomes have withdrawn from the pole. Note spindle fibers to the two still near the pole, indicating they are centric chromatids. Benda, alizarin-crystal violet.

FIGURE 49. *Lioplax subcarinata*. Entire cell in late anaphase. Most of the chromosomes are moving away from the poles. They are moving within the spindle and parallel with its fibers. (The notch at the upper border of the equatorial region is not the beginning of the telephase cell constriction but is a distortion due to the pressure of an adjacent cell.) Hermann, gentian violet, thick section.

FIGURE 50. Slightly later than 49. *Lioplax*. Polar view of the equatorial region. 44 of the total of 52 (4N) chromatids are in the equatorial plate region as a result of anaphasic counterpole movement. Hermann, gentian violet.





POLLISTER CENTRIOLE AND CENTROMERE

PLATE 4

All cells are of the atypical series.

FIGURE 51. *Lioplas*, side view of the same stage as FIGURE 50. All but 5 of the chromatids are at the equator. Hermann, gentian violet.

FIGURE 52. *Viviparus malleatus*. Early telophase. Centrioles dividing at this stage as shown by their smaller size (compare with FIGURES 41, 44, 46) and their greater number. It is also at this stage that the centriole of the typical primary spermatocyte divides (FIGURE 19). Hermann, hematoxylin.

FIGURE 53. Late telophase I. One small nucleus in each secondary spermatocyte. Most of the chromatids have remained separate and as yet unchanged. One chromatid near the nucleus in the right cell has become a vesicular body, with the chromatin in a mass at one side. Eventually all the 32 chromatids that have not been included in the normal nucleus will become vesicles, in which condition they remain until their disappearance early in spermiogenesis. Hermann, hematoxylin.

FIGURE 54. Late telophase. Centriole group opening up. Sure counts of the number of centrioles are difficult, but it is easily seen that there are approximately twice as many as during the early part of the first maturation division. Hermann, hematoxylin.

FIGURE 55. Slightly later than 54, showing the centrioles are moving apart. Note normal nuclei, and several vesiculated abnormal chromosomes in each cell. Hermann, hematoxylin.

FIGURE 56. Slightly later than 55. A focal plane through the center of the cell, showing a small aster about each centriole. Chromosomes and nucleus omitted from this drawing. Hermann, hematoxylin.

FIGURE 57. Slightly later than 56. May be classed as early prophase II, although spindle remnant of first division is still present. Left cell drawn at focal plane through center of the cell; right cell drawn to show upper surface. Chromosomes omitted. The centrioles, each with an aster, are now all on the cell periphery. Hermann, hematoxylin.

FIGURE 58. Later prophase II. Centrioles are aggregating; each group with a single large aster. This figure shows clearly the structure of the vesiculated chromatids. Hermann, hematoxylin.

FIGURE 59. Later prophase II. Centrioles are now in two groups at opposite sides of the cell, in their final position for division II. Hermann, hematoxylin.

FIGURE 60. Polar view of one group of centrioles and its aster at stage of FIGURE 59. Hermann, hematoxylin.

FIGURE 61. Second division, anaphase. One chromatid at each pole; the abnormal chromatids do not move to either pole. Benda, alizarin-crystal violet.

FIGURE 62. Same stage as 61, but showing the spindle better. The spindle is not at first continuous across the cell, but instead a cone is developed from each group of centrioles. The heavy curved lines near the spindle are probably fragments of the membrane of the normal nucleus. Note that one pole has more centrioles than the other. Benda, alizarin-crystal violet.

FIGURE 63. Early telophase II, complete cell. From the position of the cleavage furrow it is evident that all the abnormal chromosomes will be included in the larger daughter cell at the right. Hermann, gentian violet.

FIGURE 64. Anaphase II, showing the perfect orientation, V-shape, and the half-spindles of the two normal chromatids. Benda, alizarin-crystal violet, from a slide made by Miss Marie Holtz.

FIGURE 65. Telophase II. All abnormal chromosomes and one normal chromosome in spermatid at left; one normal chromosome and nearly all mitochondria (shown here as a general granulation) in cell at right. Hermann, hematoxylin.

FIGURE 66. Early spermatid, showing normal nucleus and abnormal chromosomes before any of the latter have disappeared. Hermann, hematoxylin.

PLATE 5

Figures 67-79 are various stages in the spermiogenesis of *Viviparus malleatus*. Figures 80-82 show the chromosomes at first division in three other species

FIGURES 67-68 Successive stages in the disappearance of the abnormal chromosomes Hermann, gentian violet.

FIGURE 69. Late telophase II. The smaller spermatid, at the left, has a smaller centriole group and nearly all of the mitochondrial granules. Each centriole has enlarged, elongated vertical to the cell surface, and developed a flagellum. Benda, alizarin-crystal violet.

FIGURES 70-71. Small and large spermatids, slightly later than FIGURE 69. Nucleus has chromatin in what corresponds to the shell pattern of normal spermatids (FIGURE 26). This is just before division of the centriole into proximal and distal parts. Benda, alizarin-crystal violet.

FIGURES 72-73. Later large and small spermatids, showing proximal and distal centrioles. Benda, alizarin-crystal violet.

FIGURES 74-75. Same stage as 72 and 73, but looking down on the group of proximal centrioles; nucleus seen behind the centriole group. It is at this stage that most of the centriole counts were made. Benda, alizarin-crystal violet.

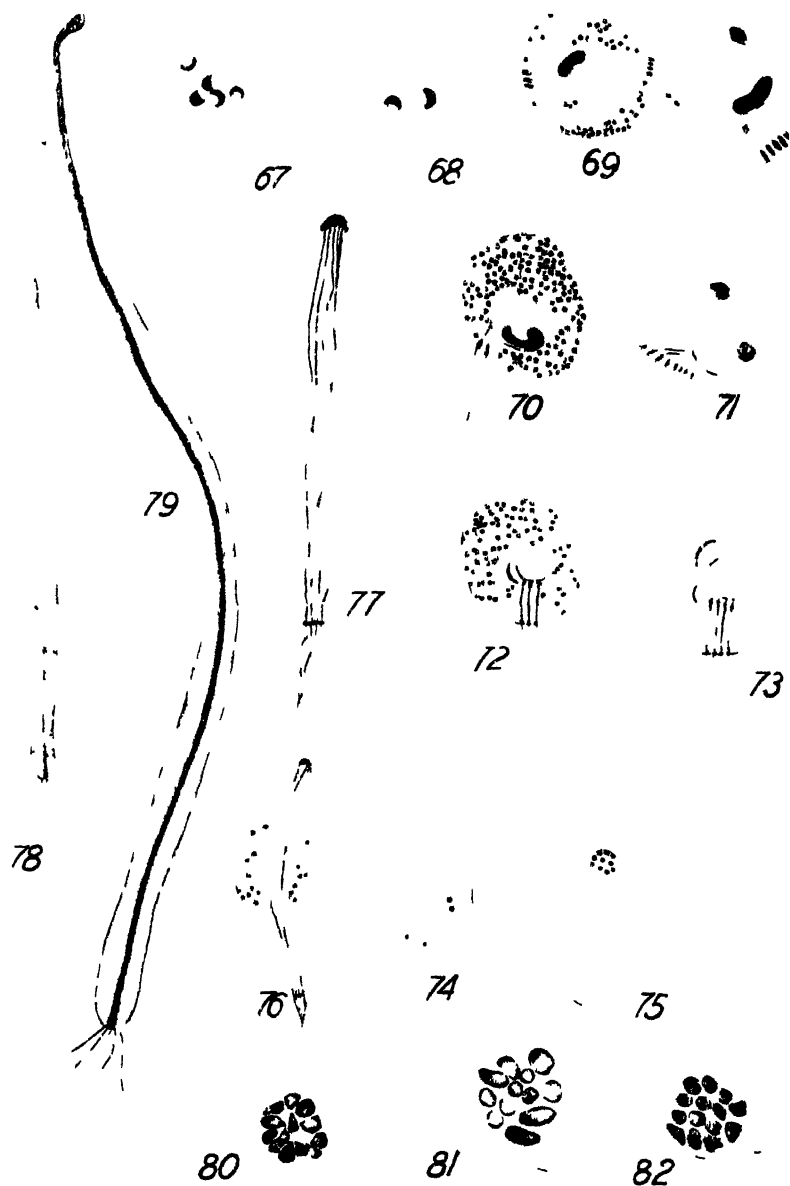
FIGURES 76-77. Later small and large spermatids. Hermann, hematoxylin

FIGURES 78-79. Nearly mature small and large spermatids. The former still appear granular from the content of mitochondria. Benda, alizarin-crystal violet

FIGURE 80. *Loiplax subcarinata*. The 13 tetrads. Hermann, hematoxylin.

FIGURE 81. *Tulotoma magnifica*. The 12 tetrads. Hermann, hematoxylin.

FIGURE 82. *Campeloma ponderosum*. The 14 tetrads. Hermann, hematoxylin.



POLLISTER CENTRIOLE AND CENTROMERE

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
VOLUME XLV, ART. 2. PAGES 49-130
DECEMBER 29, 1943

LYCAENIDAE OF THE ANTILLES*
(LEPIDOPTERA, RHOPALOCERA)

By

WILLIAM P. COMSTOCK AND E. IRVING HUNTINGTON

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* Publication made possible through a grant from the income of the Nathaniel Lord Britton Fund.

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INTRODUCTION

In the collections of The American Museum of Natural History, we found available for study from the islands of the West Indies a large number of undistributed Lycaenidae accumulated over a period of years, the result of many field trips by the staff and of miscellaneous accessions. The name species, as usually recognized from the Antilles as a whole, were well represented. A purely taxonomic study of this material was undertaken for the purpose of checking the determination of the species and incorporating the specimens in the classified collection. Our mutual approach was at first casual, for we both thought we had some knowledge of the Lycaenidae of the islands; at least we had been calling certain forms by certain names for years, and our names for them were those usually accepted by most of the authors.

Upon sorting some thousands of specimens and grouping them by species with each island population separated, we became aware of differences heretofore unnoted, which were of both specific and subspecific significance. There were more species and subspecies than we could account for from the various local island lists, and we decided upon a search of the literature and a careful survey of the Antillean Lycaenidae.

It was at once obvious that certain names of continental species were involved, either through the actual occurrence of the species in the islands, or through the misapplication of names. This extended the work, but also served to define more clearly what species actually occur in the islands. The region considered includes the Bahamas and the Greater and Lesser Antilles. Certain forms occurring in southern Florida were included in this study because of their Antillean relationships. Trinidad was included with the continental area, which was considered only to the extent that it became necessary to mention species related to Antillean species or which were wrongly listed from the Antilles.

We were impressed with the paucity of information about the Antillean Lycaenidae. A review of the literature showed that Drury was the first author to describe an Antillean species. He was followed by Fabricius, Cramer, Hübner and Godart; and, at a later period, Poey, Lucas, Hewitson and Herrich-Schäffer described a number of species. In the last hundred years about a score of authors have discussed the Antillean Lycaenidae, for the most part, briefly. The localities given by most of these authors needed careful consideration, for some are indefinite, others inaccurate, and occasionally no locality was given. Because of indefinite localities and misapplication of names to Antillean butterflies by various authors, considerable confusion existed in

the literature. This we have tried to correct. In some cases, the results obtained are more expressions of our personal views than proof, but we have endeavored to use and preserve existing names even when in doubt, rather than to describe the forms as new species. The names of *Lycænidæ* attributed to the Antilles make an extensive list, and we have set down all that we encountered in the literature.

Although we have studied what, at first sight, appeared to be a large number of specimens, we feel that it represents but a cross section of the fauna. There is need for much more thorough collecting as well as field study on the life histories, of which, it might be said, practically nothing is known.

We recognize 62 distinct forms, consisting of 36 species with an additional 26 subspecies, occurring in southern Florida and the Antilles. We have examined all of them except the following four described by Clench: *Thecla angelia dowi*, *Hemiargus ammon thomasi*, *Hemiargus bahamensis*, and *Brephidium barbouri*. We have consistently determined all species from the original descriptions and figures, and we have checked as fully as we were able the determination of other authors who have listed Antillean species, correcting these determinations to the best of our ability. Of the 36 Antillean species, 4 are described as new, and, of the 26 additional subspecies, 18 are described as new.

The classification used follows approximately the order of Draudt:

THECLINAE

Eumæus

Thecla

PLEBEJINAE

Hemiargus

Brephidium

Leptotes

All names of species, apparently incorrectly attributed to the Antilles, are marked with an asterisk (*). Certain other species, not known from the Antilles, included because of their close relationships to Antillean populations, are placed in taxonomic sequence in the body of the text. These also are marked with an asterisk (*).

All types, except as otherwise mentioned, are in the collection of The American Museum of Natural History.

The terms used in descriptions of species are explained by the accompanying diagrams of the wings of the Theclinae and Plebejinae. In the Antillean Theclinae, vein R_4 in the forewing is absent. Tails are frequently present at both Cu_1 and Cu_2 . The two marginal spots occur in the hindwing of many Plebejinae as well as Theclinae and the mesial line is manifested in various forms in both subfamilies. The veins are frequently referred to in our descriptions to locate markings

and the expression "above Cu_2 " means between veins Cu_1 and Cu_2 . The length of a spot is considered to be its extent from the margin basad and the breadth of a spot is considered to be its extent parallel with the margin. The discal cell, which is the only closed cell of the wing, is termed cell. The length of the forewing is measured from the base to the apex.

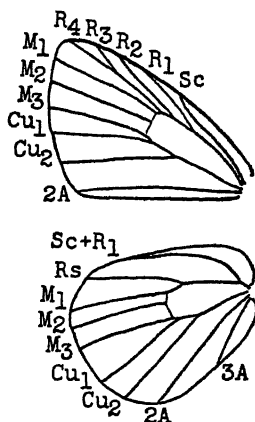
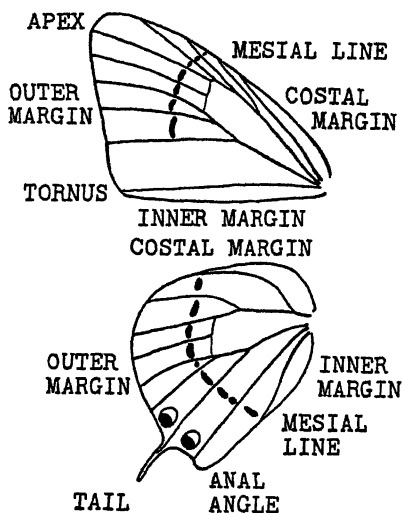


FIGURE 1. Conventional wings of Theclinae.

FIGURE 2. Conventional wings of Plebejinae

We have had valued assistance from Dr. A. Avinoff of the Carnegie Museum in making this study of the Antillean Lycaenidae. He has examined the species we have determined and described, and aided us by his keen observations. The loan of specimens for study from his fine Jamaican collection is also acknowledged.

We wish also to acknowledge the kind assistance of Mr. Nathan Banks of the Museum of Comparative Zoölogy and of Dr. W. T. M. Forbes of Cornell University, both of whom have loaned specimens for study. We are greatly indebted to Dr. C. D. Michener, of the American Museum staff, for general assistance and careful criticism of the manuscript, and also to Miss Annette L. Bacon, Miss Alice Gray and Miss Lucy Clausen for their interest and help in the preparation of this paper. The photographs for the plate were made by the Photographic Division of The American Museum of Natural History.

UNRECOGNIZED AND EXTRALIMITAL SPECIES

The following names recorded from the Antilles by certain previous authors have either not been recognized by us or the species they represent are known from some other area and are believed not to occur in the Antilles.

* *Thecla endymion* (Fabricius)

Papilio endymion Fabricius (1775) 519, No. 324. Brazil.
Polyommatus hugon Godart (1822) 640, No. 84. No locality.
Thecla hugo Doubleday (1847) Part 2, 32. Brazil, Pernambuco.
Thecla hugo, Westwood (1852) 486, pl. 74, fig. 4. Brazil.
Imolus endymion, Butler (1866) 188. Para.
Thecla endymion, Draudt (1920) 706, pl. 158, c. Colombia.

Thecla endymion (Fabricius) does not occur in the Antilles nor in Florida to our knowledge. It does occur in eastern Brazil, and we have so identified a series of both sexes from Igarapi Assú, near Pará, Brazil. Others also have determined it as a Brazilian species from the same general region. Godart gave no locality for his *hugon* but his description will pass for a male of *endymion*. We are indebted to Mr. C. F. dos Passos for a photograph of a specimen in the Paris Museum, which M. Le Cerf considered the type of *hugon*. This specimen is without a pin label and is a female. It cannot be the type but, judging from the photograph, it is a female of the species we recognize as *endymion*. The name *hugo* Doubleday we consider a misspelling of *hugon* and we feel sure that the figure of *hugo* (Westwood, 1852) represents *endymion*.

The use of the name *hugon* for a Cuban species and hence its introduction into North American lists, we believe originated with Herrich-Schäffer (1862: 142), who misidentified *angelia* from Cuba under the name *hugo*.

Holland (1931: 242, pl. 64, fig. 32) figured as *endymion* a specimen from Haiti which is incorrectly determined, for, having examined the specimen, we know this to be an undescribed subspecies of *angelia*. The matter becomes the more confused because Holland credited *endymion* to Cramer. The *endymion* of Cramer is a species of the family Riodinidae now placed in the genus *Helicopsis*. Incidentally, *Papilio endymion* Cramer is a homonym of *Papilio endymion* Fabricius and it is at present improperly used.

Draudt's figures are not of typical *Thecla endymion* occurring in eastern Brazil. In his text Draudt gave the locality Colombia for *endymion* and, if the specimen figured came from there, it perhaps represents a subspecies intermediate between *endymion* and *cyphara* Hewitson, which was described from Panama and ranges as far as Mexico. We omit the further and quite complicated synonymy of *endymion* as beyond the scope of the present discussion.

* *Thecla ixion* (Fabricius)*Papilio ixion* Fabricius (1775) 523, No. 340. "In India."*Strymon ixion*, Butler (1869) 190.*Thecla ixion*, Kirby (1871) 398, No. 299. Antilles.

Butler mentioned a male from Oaxaca, Mexico, in the collection of the British Museum. Kirby gave the locality "Antilles." Draudt (1921) did not mention the name.

* *Thecla euripedes* (Fabricius)*Hesperia euripedes* Fabricius (1793) 267, No. 32. "In Indiis."*Strymon euripedes*, Butler (1869) 190.*Thecla euripedes*, Kirby (1871) 398, No. 299. Antilles.

Fabricius gave the reference "Jon. fig. pict. 6. tab. 13. fig. 4." to Jones "Icones." Butler placed the name in synonymy as the female of *ixion* (Fabricius) and said it came from Oaxaca, Mexico. Draudt (1921) did not mention the name.

* *Thecla anacreon* (Fabricius)*Hesperia anacreon* Fabricius (1793) 268, No. 34. "In India."*Strymon anacreon*, Butler (1869) 191.*Thecla anacreon*, Kirby (1871) 398, No. 300. Antilles?

Fabricius gave the reference "Jon. fig. pict. 6. tab. 5. fig. 4." to Jones "Icones." Butler said that it was not in the British Museum. Kirby appended a question mark to his locality. Draudt (1921: 825) did not know the species.

* *Thecla inachus* (Cramer)*Papilio inachus* Cramer (1775) 1, 58, pl. 36, D. "Indes Occidentales."*Thecla inachus*, Draudt (1920) 750, pl. 148, c.

This South American species occurs over an extended range, but we do not know of an authentic record of its capture in the Antilles.

* *Thecla hyacinthus* (Cramer)*Papilio hyacinthus* Cramer (1775) 1, 59, pl. 36, E. "Indes Occidentales."*Thecla hyacinthus*, Draudt (1920) 785, pl. 156, b.

We cannot find an authentic record of this species from the Antilles, although Draudt recorded it from the West Indies.

* *Thecla ganymedes* (Cramer)*Papilio ganymedes* Cramer (1775) 1, 64, pl. 40, C. D. "Indes Occidentales."*Thecla ganymedes*, Draudt (1920) 746.

We are in doubt as to the correct determination of Cramer's species. Related forms occur in various parts of South and Central America, north to Vera Cruz, Mexico, but we know of none from the Antilles.

* *Thecla ethemon* (Cramer)*Papilio ethemon* Cramer (1775) 1, 75, pl. 48, D. "Indes Occidentales."

We know of nothing resembling this figure occurring in the Antilles or elsewhere. Draudt (1921) did not mention the name. If the tails are disregarded, the figure is not unlike *Euselasia euboica* Hewitson (1852, 4 (1): pl. 54, fig. 45).

* *Thecla ergeus* (Godart)

Polymmatius ergeus Godart (1822) 635, No. 67. Antilles.

We cannot identify this species from the original description as occurring in the Antilles or elsewhere. Draudt (1921: 825) did not know it.

* *Thecla marius* Lucas

Thecla marius Lucas (1857) 599. Cuba.

Thecla marius, Gundlach (1881a) 114.

Lucas said that this was similar to *syncellus*, but we do not know of anything like it from the Antilles. Gundlach suggested that it might be *limeria* Hewitson, but this is evidently an error.

* *Thecla paseo* Lucas

Thecla paseo Lucas (1857) 609. Havana.

We do not know this species.

* *Thecla aon* Lucas

Thecla aon Lucas (1857) 610, pl. 16, figs. 6, ♂; 6a, b, ♀. Havana and Yucatan.

Thecla aon, Herrich-Schäffer (1864) 165.

Thecla aon, Gundlach (1881a) 114.

We have been unable to determine this species. Gundlach said that he had not observed it and that Herrich-Schäffer misdetermined *celida* as *aon*.

* *Thecla tollus* Lucas

Thecla tollus Lucas (1857) 611. Cuba.

Thecla tollus, Gundlach (1881a) 114.

Lucas said that it was similar to *strephon*, but we have been unable to recognize it, although the description applies very well, in part, to *coelebs* Herrich-Schäffer. Gundlach suggested that it might be *martialis* Herrich-Schäffer. Draudt (1921) did not mention the name.

* *Thecla ergina* Hewitson

Thecla ergina Hewitson (1867) 105, No. 137, pl. 43, figs. 170, 171. Jamaica.

Thecla ergina, Draudt (1920) 769, pl. 152, c.

This species is not known to us from the Antilles proper, but specimens from Trinidad, Venezuela and near Pará, Brazil, agree with the description and figures.

* *Thecla cardus* Hewitson

Thecla cardus Hewitson (1874) 151, No. 264, pl. 60, figs. 394-396. Brazil.

Thecla cardus, Dewitz (1877) 241.

Thecla cardus, Draudt (1920) 808, pl. 159, i, k.

Dewitz employed this name in his list of species from Puerto Rico, giving the reference to Hewitson's plate and figures. He undoubtedly had an Antillean species very close in appearance to those figures, but we believe his determination was incorrect. We recognize a species

of similar appearance from Hispaniola, but we have not seen it from Puerto Rico. This is described below, as a new species, under the name *christophei*.

* *Tarucus ubaldus* (Cramer)

Papilio ubaldus Cramer (1781) 4, 209, pl. 390, L. M. Coromandel.

Polyommatus ubaldus, Godart (1822) 652, No. 204.

Cupido ubaldus, Kirby (1871) 350, No. 67.

Tarucus ubaldus, Fruhstorfer (1923) 894, pl. 153, e.

The inclusion of this name in the synonymy of *hanno* (Stoll) seems to have originated with Kirby whose reference reads: "*Pol. Ubaldus*, Godt. (nec Cram.) Enc. Méth. IX. p. 682. n. 204 (1823)." Thus Kirby, for some reason, concluded that Godart referred to some other species than *ubaldus* (Cramer). We cannot see that this is so, for Godart accepts Cramer's locality "Coromandel" and there is nothing in his description that is in disagreement with Cramer's figures. We feel that Kirby's action was unwarranted and that *ubaldus* should be removed from the synonymy of *hanno*.

* *Tarucus plinius* (Fabricius)

Haesperia plinius Fabricius (1793) 284, No. 92. "In Indiis."

Papilio plinius, Donovan (1800) pl. 45, fig. 1.

Lampides plinius, Butler (1869) 164. Jamaica.

Cupido plinius, Kirby (1871) 351, No. 75. India, Java.

Tarucus plinius, Fruhstorfer (1923) 893, pl. 153, b. Indo-Australian region.

While Butler recognized something, probably *theonus*, as this species from Jamaica, Kirby identified *plinius* as an Indo-Australian species.

* *Leptotes ochsenheimeri* (Godart)

Polyommatus ochsenheimeri Godart (1822) 683. "Antilles?"

Cupido ochsenheimeri, Kirby (1871) 349, No. 50.

Leptotes ochsenheimeri, Draudt (1921) 821.

This description fits no Antillean species, but suggests a species belonging to an Andean group of *Lycacnidae*. Godart appended a question mark to his locality.

* *Lycaena hamo* Lucas

Lycaena hamo Lucas (1857) 612.

Lucas attributed this name to Hübner, giving the reference: "Hübner, Samml. exot. Schmett." Since this name was not used by Hübner, Lucas is its author. Although *hamo* has been placed heretofore in the synonymy of *hanno* (Stoll), we can see no justification for this action. The statement, "fimbria in utroque sexu intersecta" (fringe in both sexes cut apart, i.e. interrupted), would definitely separate it from *hanno*. We do not recognize this species from the Antilles, or elsewhere.

* *Lycaena astenidas* Lucas

Lycaena astenidas Lucas (1857) 613.

We cannot determine any Cuban or other Antillean butterfly from

Lucas' description. The name has been placed, usually, in the synonymy of *hanno* (Stoll), but we do not feel justified in so doing.

In additional comment on the last two of Lucas' names, we note that Lucas also recorded *hanno* (1857: 614). He attributed *hanno* to Hübner, giving the same Sammlung reference as he gave for *hamo*. He also gave the Stoll reference and placed *filenus* (Poey) and *pseudoptiletes* (Boisduval and LeConte) in synonymy. His description defined *filenus* very well except for size, which he gave as 35 mm. expanse for the male and 37 mm. for the female. In more than fifty specimens of *filenus* examined, the largest had a length of forewing of 11.4 mm., which would correspond to an expanse of 25 mm. at the most. Other factors considered, we suggest that Lucas was in error in his dimensions, but that the butterfly he described was *filenus*.

The listing of Lycaenidae by Lucas in Sagra's "Histoire de L'Isle de Cuba" (1857: 593-617) has undoubtedly been a great source of confusion, because some of the species he listed in error were quoted by later authors.

Alexandre Lefebvre had undertaken a study of the Lepidoptera of the Antilles before 1838, but did not continue the work. Lefebvre's specimens were used by Lucas twenty years later for his work on Cuban Lepidoptera. Unfortunately, this material contained many species which did not occur in Cuba or even in the Antilles. Gundlach (1881a: 114) commented on this material, presumably unlabeled, and confined his own list to the species which he knew to occur in Cuba. For the information of the student who may not have access to "Sagra," we list the species cited by Lucas in the manner and order in which he presented them. The dagger (†) indicates the species we recognize as Antillean, all of which are further discussed.

Eumenia

† *atala* Poey

Thecla

† *marsyas* Linnaeus

endymion Fabricius

endymion regalis Swainson, Cramer

halesus Cramer

meton Cramer

irus Godart

lincus Fabricius

synonym *aetolus* Cramer

† *simaethis* Drury

hemon Fabricius

lausus Cramer

synonym *libanius* Cramer

- marius* Lucas
- narbal* Stoll
- sichaeus* Cramer
- echion* Linnaeus
- eurytulus* Hübner
- † *mars* Fabricius
 - † synonym *acis* Drury
 - synonym *ixion* Fabricius
- † *beon* Cramer
 - m-album* Boisduval and LeConte
 - arsace* Boisduval and LeConte
 - damon* Cramer
 - synonym *smilacis* Boisduval and LeConte
 - calanus* Hübner
 - synonym *jalacer* Godart
 - javonius* Abbot and Smith
 - liparops* Boisduval and LeConte
- † *bubastus* Cramer
 - † synonym *columella* Fabricius
- megurus* Godart
- niphon* Hübner
- paseo* Lucas
- aon* Lucas
- † *celida* Lucas
- tollus* Lucas

Lycaena

- † *theonus* Lucas
- † *ammon* Lucas
 - hamo* Fühner
- astenidas* Lucas
- † *cassius* Fabricius
- † *hamo* Hübner
 - † synonym *filenus* Poey
 - synonym *pseudoptiletes* Boisduval and LeConte

Chrysophanus

- thoe* Boisduval and LeConte
- epixanthe* Boisduval
- tarquinius* Fabricius
 - synonym *crataegi* Boisduval and LeConte

SYSTEMATIC ACCOUNT

Species known to occur in the Antilles, together with some observations on the related continental species.

Theclinae

EUMAEUS Hübner

Eumaeus Hübner (1813) Verzeichniss, 67, No. 643.

Eumenia Godart (1823) 826.

Genotype.—*Rusticus Adolescens minijas* Hübner, 1809.

Eumaeus atala atala (Poey)

Eumenia atala Poey (1832) Cuba.

Eumenia torea, Gray (not Godart) (1832) 787 (*tozia*), pl. 43, fig. 3 ("*tozea* Godart").

This species was excellently figured and clearly described by comparison with *minijas* Hübner. The name *Eumenia torea* Gray is a

misidentification of *Eumenia toxea* Godart (1823: 826), which is considered a subspecies of *Eumaeus minijas* Hübner by Lathy (1926: 39). This black-winged species, with its metallic blue or green suffusion and spotting, and red abdomen, is well known. The form occurring in Florida has usually gone under the same name, but it is there represented by a separable subspecies.

Gundlach (1881: 80) gave the food plant "*Cycas revoluta*." Also see Dethier (1941: 75).

* *Eumaeus atala grayi* NEW SUBSPECIES

Eumaeus atala, Holland (1931) 223, pl. 28, fig. 22, ♂.

In size and shape, ground color and markings, specimens from Florida correspond with *atala atala* from Cuba, except in the following ways: in males, on the upperside of the forewings, the blue-green metallic scaling extends farther distad, causing a narrower black border, and there is less black scaling along veins; in the hindwing, the marginal metallic spots are at least twice as long as in *atala* and broadened so as to be nearly confluent and only the posterior half of the cell is green (in *atala* the cell is usually fully green); on the underside of the hindwings the spots of the three rows of metallic spots are considerably larger than in *atala*. In females, on the upperside of the forewings, the metallic scaling is more extended above the inner margin; the marginal metallic spots of the hindwings are enlarged and, on the underside of the hindwings, the metallic spots are increased in size, as in the males.

Types, all from Florida: holotype, male and allotype, female, Miami, February 10 and 6, 1916, collector, W. L. Haskin. Paratypes: from Miami, ♀, January 27; 3 ♀, February 6; ♂, February 10; ♀, February 27; ♀, April 11-21; 4 ♂, 3 ♀, November 5; 5 ♂, no date; ♂, 3 ♀, Biscayne Bay, no date, and ♂, no data (A. T. Slosson); ♂, 3 ♀, Homestead, April 18; ♂, ♀, Royal Palm Park, March 25..

The following fifteen paratypes, all from Miami, Florida, are in the collection of Mr. C. F. dos Passos: 4 ♂, 4 ♀, February 6, 1916; ♂, February 27, 1910; ♂, February-March 1903; 2 ♂, ♀, no date; 2 ♀, 1930.

One paratype is in the collection of Mr. Harry K. Clench: ♀, Long Pine Key, Florida, February 3, 1937.

In the collection of the Museum of Comparative Zoology, Cambridge, Massachusetts, there are the following paratypes: 4 ♂, ♀, Cocanut Grove, Florida; ♂, 3 ♀, Long Pine Key, Dade Co., Florida, January, 1931; ♀, Miami to West Palm Beach, Florida, April 6, 1925.

In the collection of Cornell University, Ithaca, N. Y., there are the following paratypes: 4 ♂, 2 ♀, Miami, Florida, February-March,

1903, collector J. H. Comstock; ♀, Miami, Florida, February 7, 1919; ♀, Cocoanut Grove, Florida, February 11, 1905.

This subspecies is named for the entomologist, George Robert Gray (1808-1872).

THECLA Fabricius

Thecla Fabricius (1807) x.
Thecla, Illiger (1807) 6, 286.
Thecla, Hemming (1934) 115.
 Genotype.—*Papilio betulae* Linnaeus, 1758.

We have used the generic name *Thecla* in a broad sense, to include all of the Antillean Theclinae other than those of the genus *Eumaeus*.

Thecla marsyas cybele Godman and Salvin

Thecla cybele Godman and Salvin (1896) 516. St. Vincent.
Thecla marsyas cybele, Draudt (1919) 748.

This well-defined subspecies is easily distinguishable from continental *marsyas* by the deeper blue of the upperside and the greater extension of the apical and marginal black areas, which cover almost half of the forewing in both sexes. On the underside, the purplish tone is stronger than in typical *marsyas* and there is a decided blue-green frosting; the black maculation is heavier. We have seen a pair from St. Vincent, B. W. I.

Thecla coelebs Herrich-Schäffer

Thecla coelebs Herrich-Schäffer (1862) 142. Cuba.
Thecla coelebs, Druce (1907) 594 (part).
Thecla coelebs, Draudt (1920) 779, pl. 155, f. g.

Herrich-Schäffer described this species from Cuba, differentiating it from *angelia* Hewitson (1874), which he misdetermined under the name *hugo*. We have seen *coelebs* from Cuba only, and we believe it to be endemic in that island and doubt its occurrence elsewhere.

Gundlach (1881: 75) gave some life-history information.

Thecla bourkei Kaye

Thecla bourkei Kaye (1924) 416. "Trinidad."
Thecla bourkei Kaye (1931) 336, pl. 59, fig. 8, ♂. Jamaica.

Kaye, in his original description, gave the locality for *bourkei* as Trinidad, but later corrected this to Jamaica. We have studied one male from Jamaica which agrees with the description and the later published figure. The species may be restricted to Jamaica.

Thecla fidena Hewitson

Thecla fidena Hewitson (1867) 112, No. 159, pl. 44, figs. 183, 184. No locality. (Puerto Rico and Hispaniola.)
Thecla coelebs, Druce (not Herrich-Schäffer) (1907) 594, part.

This species which occurs in Hispaniola and Puerto Rico has been heretofore erroneously determined as *coelebs*. A pair from Hispaniola

and a single male from Puerto Rico agree with Hewitson's figures of *fidena* and we are thus able to determine this species.

The three species just discussed are easily separated, for *bourkei* has, in males, a very distinct stigma in the forewing cell and lacks the hair tuft near the base of Sc of the hindwing, characteristic of both *coelebs* and *fidena*. In *coelebs*, the tail at Cu₁ is developed, but in *fidena* it is obsolete; the two differ definitely in underside pattern. We do not agree with Druce that *fidena* is the male of *coelebs* and a synonym. The species which we recognize as *fidena* from Hispaniola and Puerto Rico is abundantly distinct from *coelebs* of Cuba in both sexes.

Thecla piplea Godman and Salvin

Thecla piplea Godman and Salvin (1896) 516. St. Vincent.

Thecla subobscura Lathy (1904) 452. Dominica.

Thecla piplea, Draudt (1920) 780.

Draudt placed *subobscura* as a synonym of *piplea*. We recognize as this species a pair from St. Vincent, the type locality of *piplea*, and three males and a female from Dominica, the type locality of *subobscura*. The material is insufficient to pass judgment upon the correctness of the synonymy, but the specimens from the two localities are certainly very much alike.

Thecla beon (Cramer)

Papilio beon Cramer (1780) 4, 61, pl. 319, B, C. Surinam.

Thecla beon, Hall (1925) 189.

We include this species among the Antillean fauna on the authority of Hall, who recorded "Two or three on Mt. Isabella" in his list of the butterflies of Hispaniola. We know the species from South and Central America, but not from the islands.

* *Thecla cecrops* (Fabricius)

Hesperia Rurales cecrops Fabricius (1793) 270, No. 41. "In Indiis."

Calycopis cecrops, Field (1940a) 183.

We list this species because it is common in southern Florida, whence its range extends northward into the Atlantic coast states and westward into the Gulf states. Its synonymy and relationships to other similar, widespread continental species, such as *beon*, would involve an extensive study considered beyond the scope of this paper.

Thecla acis acis (Drury)

Papilio acis Drury (1770) 1, 2, pl. 1, fig. 2. "New York . . . 31st. of August."

This species seems to be poorly understood. Drury's description is sufficiently clear to define what he had before him, but in each of four copies of his "Illustrations of Exotic Entomology," which have been ex-

amined, the figures are different and only in one does the figure agree with the original description which reads in part:

"Under-side.—The *Palpi*, *Breast* and *Abdomen*, are white.—All the *Wings* on this side are of a dark lead colour.—A very narrow black and white line crosses the *Superior Wings*, parallel with the external edges; another indented irregular line crosses the *Inferior Wings*, beginning near the middle of the anterior edge, and meeting just below the extremity of the body.—Four long reddish spots are very visible on this side, below which are four black ones."

In the "Drury," in the Cornell University Library, there is a figure which corresponds with the description. From this and the text it is possible to determine with some precision the butterfly to which the name *acis* applies.

Because of the crudeness and dissimilarity of the figures in various copies of Cramer's work (1777, 2: 120, pl. 175, C, D), it is very difficult to say which subspecies of *acis* is represented. Cramer said that it had been taken at the Cape of Good Hope, which is even more remarkable than Drury's locality, "New York."

The specimens before us may be roughly separated into two groups of subspecies: one coming from Hispaniola, Puerto Rico, several of the Virgin Islands and Antigua; the other from Florida, the Bahamas, Cuba and Jamaica.

Specimens from Antigua seem very close to *acis* as described by Drury, but it is possible that there may be a population in some more southerly island of the Lesser Antilles which will entirely satisfy his description. For the present, we doubtfully call the population of Antigua *acis acis*, but we feel that it may prove to be a closely related subspecies. Lathy (1904: 453) recorded "*acis*" from Dominica, but we have not seen any specimens from this island.

Thecla acis mars (Fabricius) NEW COMBINATION

PLATE 1, FIGURE 5

Papilio mars Fabricius (1777) 26b, No. 321-25. "America meridionali."
Strymon mars, Hübner (1823) Sammlung, 2, pl. [89], figs. 1-1.

This species, as described by Fabricius and later figured by Hübner, we recognize as a subspecies of *acis* occurring in several of the Virgin Islands. The population of Puerto Rico we class with these at present. Virgin Island specimens differ from *acis acis*, as it was described, as follows: on the underside of the hindwing, distad of the mesial line, there is an irregularly triangular orange patch based on Cu_2 and with its apex at Rs ; in many specimens this orange area is prolonged distad along veins Cu_1 and Cu_2 almost to the margin, outlining a lunule which

is gray within and separated from the orange by a line of black scales. (In *acis acis* at this place there is a large black spot, an approach to which appears in specimens from Antigua, which also, like *acis*, do not show the prolongations of the orange color along the veins.) The anal lobe is heavily black-scaled in both *mars* and *acis*, but the area basad of the lobe along the inner margin is white-scaled in *mars*, with only traces of orange scales basally (in *acis* this area is orange nearly to Cu_2). In specimens from Antigua, the width of the orange-scaled area is greater, thus approaching *acis acis*, and the orange color is more reddish.

A very careful comparison of specimens from St. John and St. Thomas, V. I., with the original "Sammlung" figures convinces us that it was such material that Hübner had before him and figured as *mars*. We feel that this is the true *mars* of Fabricius. The figures in the Westwood reprint edition of Hübner's work are poor reproductions and of little value.

We have examined a female loaned by Cornell University which was taken at Basse Terre, St. Kitts, B. W. I., March 26, 1927. This agreed exactly with the Virgin Island series.

In Hispaniola, there is another subspecies of *acis* which is intermediate between the northern and southern groups of subspecies, but we think it is nearer the latter.

Thecla acis petioni NEW SUBSPECIES

PLATE 1, FIGURE 4

Size and shape: the length of forewing is 13.4 mm. in three specimens. The wings are shaped as in *mars*.

Ground color: this is black-brown on the upperside and clear gray on the underside, as in *mars*.

Markings: on the underside, the pattern is similar to that of *mars* but differs as follows. In the hindwing, the orange patch extends scarcely beyond M_3 and is both narrower and shorter than in *mars*; the narrow submarginal white line starts at Cu_1 and is clearly defined as far as Rs , not being suffused distad; the mesial line continues beyond Cu_1 , bending slightly basad (whereas in *mars* it stops on Cu_1 and is set slightly distad).

Types, all from Port-au-Prince, Haiti: holotype, male, January 1-6, 1922; allotype, female, January 13, 1922. Paratype: ♀, January 13, 1922.

This subspecies is named for Alexandre Sabes Pétion (1770-1818), Haitian general and patriot.

Of the second group of subspecies, the population from Florida is the best known and is very distinctive.

* *Thecla acis* *bartrami* NEW SUBSPECIES

PLATE 1, FIGURE 2

Thecla acis, Draudt (not Drury) (1920) 798, pl. 138, e.*Thecla acis*, Holland (not Drury) (1931) 236, pl. 20, fig. 38, ♀.

Size and shape: the length of forewing varies from 12 to 14.4 mm. with an average of 13.4 mm. The wingshape and length of the tails is about the same as in *acis* and *mars*.

Ground color: on the upperside the black-brown color is about the same as in other subspecies, but on the underside the color is brown or grayish brown, not a clear gray as in the subspecies of group one.

Markings: on the underside, the forewing has a black mesial line edged distad with white, as in the other subspecies; the hindwing has a similar mesial line which is cleft at Cu_2 usually with red scales within the angle and/or below it before the line extends to the inner margin; the colored patch is red-orange and extends only from Cu_2 to slightly beyond M_3 ; distad of the red-orange patch above vein Cu_1 is usually a small rounded black spot on the ground color; submarginally beyond the red-orange patch, extending from M_3 to Rs , meeting the margin, is a white band 1 mm. wide and of nearly even width; adjoining this basad is a black line running from Rs to meet the mesial line at Cu_1 ; the anal lobe and the marginal space above 2A are black, the latter scattered with blue scales; basad of the lobal area is a white patch, then a black line, and then a more or less gray-scaled space distad of the mesial line, where it bends to the inner margin; near the base, in the cell and above Rs , there are the usual two white spots edged distad with black. These characters separate the Floridian population from subspecies of the first group, *acis*, *mars* and *petioni*, all of which have a differently shaped orange patch and a much narrower white area beyond it.

Types, all from Florida: holotype, male and allotype, female, Miami, August 2 and 12, 1929. Paratypes: ♂, ♀, April 11–21, 1923; 2 ♂, 3 ♀, April 15–May 3, 1904; ♂, ♀, June 15–July 2, 1904; ♀, August, 1904; ♀, December 25, 1929 (all Miami); ♂, Biscayne Bay; ♂, Lake Worth; ♂, Palm Beach; 2 ♂, ♀, March, 1921, ♀, March, 1931, Jupiter.

The following sixteen paratypes are in the collection of Mr. C. F. dos Passos: ♀, northwest section of Miami, July 20, 1939; all others are from the pinelands of Brickell Hammock, Miami, ♂, June 16, 1939; 6 ♂, ♀, July 18, 1939; ♂, 2 ♀, July 29, 1939; 4 ♂, August 18, 1939.

One paratype is in the collection of Mr. Harry K. Clench: ♂, Goulds, Florida, July 20, 1937.

In the collection of the Museum of Comparative Zoology, Cambridge,

Massachusetts, there are the following paratypes: ♂, 4 ♀, Big Pine Key, Florida, July 1, 1934.

With the series from Big Pine Key there was one more specimen (♀), which is a most interesting aberration of *bartrami*. Normal in appearance on the upperside, the underside is very unusual. On the forewing, the white distal edging of the mesial line is 1.3 mm. wide at the costa and then tapers posteriorly to a point before reaching Cu_2 ; on the hindwing, the white distal edging of the mesial line fills the entire space distad to meet the black submarginal line, thus forming a solid triangle of white in this space. All white markings of the pattern are exaggerated. There seems to be nothing about this aberration that is in any way helpful in explaining the problem of the extensive subspeciation of *acis*.

This subspecies is named for William Bartram (1739–1823), American botanist and ornithologist, who traveled in Florida during Revolutionary times.

Thecla acis casasi NEW SUBSPECIES

PLATE 1, FIGURE 1

Size and shape: the length of forewing is 11 to 12 mm.; although of somewhat smaller size, the shape is like that of other subspecies of *acis*.

Ground color: this is slightly darker brown on the upperside than *bartrami*, its closest ally; on the underside the color is a warmer brown than in *bartrami*.

Markings: on the underside, the pattern is like that of *bartrami*, except that the mesial line of the hindwing is straight from the costa to Cu_1 , not bowed or irregular; the orange patch extending from Cu_2 to M_3 is reduced in size and the color is orange, not red-orange; the submarginal white line beyond the orange patch is relatively narrower than in *bartrami*.

Types, all from Cuba: holotype, female, Santiago de Cuba. Paratypes: ♀, Santiago de Cuba; ♂, Guantánamo, Cuba, June 28, 1921; collected by and in the collection of Dr. Charles T. Ramsden, Santiago de Cuba.

This subspecies is named for Bartolomé de Las Casas (1474–1566), a Dominican monk, known as the "Apostle of the Indies."

While *bartrami* and *casasi* are closely related they are readily separated and both are distinct from *armouri* of the Bahamas.

Thecla acis armouri (Clench)

Strymon acis armouri Clench (1948) 53. Bahamas.

This subspecies from Rum Cay and Cat Island in the Bahamas has

an underside ground color of gray with scarcely a suggestion of brown. The underside pattern is quite at variance with *bartrami* and *casasi*. In the forewing, the mesial line forms a bow distad and is more narrowly edged with white than in those subspecies. The mesial line of the hindwing is also more narrowly edged in white. The orange patch is confined to the space between Cu_1 and Cu_2 , and, in front of vein Cu_1 , the black submarginal line is broad, gradually diminishing in width to Rs. Distad of this black line, the white scaling approaches the margin more closely and there is a broader white line, within the margin from 2A to M_3 , than in *bartrami* and *casasi*.

Through the courtesy of Mr. Nathan Banks, we have examined two female paratypes of this subspecies.

Thecla acis gossei NEW SUBSPECIES

PLATE 1, FIGURE 3

Size and shape: the length of the forewing is 13 to 14 mm. In shape, the wings are like those of the other subspecies of *acis*.

Ground color: this is as in *bartrami* on both surfaces; it is more brownish on the underside than in *armouri*.

Markings: this subspecies is distinguishable from *armouri* in having the mesial line of the underside of the forewing straight, not bowed distad, although the white outer edging of this mesial line is narrow as in *armouri*. In the hindwing, the orange patch is also confined to the space between Cu_1 and Cu_2 , as in *armouri*, and the line beyond is broadly black, tapering to Rs more definitely than in *armouri*; the white line distad is much more sharply defined than in *armouri*. The white marginal line is as in *armouri*, broader than in *bartrami*.

Types, all from Jamaica: holotype, female, Don Christopher's Cove, St. Ann, Jamaica, sea level, March 22, 1931, collected by E. L. Bell. Paratypes: ♀, same data; ♀, Malvern, St. Elizabeth, July 31, 1933, collected by A. Avinoff and N. Shoumatoff, and in the collection of the Carnegie Museum, Pittsburgh, Pa.

This subspecies is named for Philip Henry Gosse (1810–1888), author of "A Naturalist in Jamaica."

The subspecies *armouri* and *gossei* are geographically well-separated populations, one in the Bahamas, the other in Jamaica. While, on close examination, minute differences are evident, the two subspecies have developed along very similar lines. Quite in contrast is the intervening population of Cuba, the subspecies *casasi*, which is outstandingly different from them but close in general appearance to *bartrami* from Florida.

In summary, we arrange the described subspecies of *acis* as follows:

Thecla acis acis (Diary). Probably from Lesser Antilles. Antigua?

" " *maris* (Fabricius). Virgin Islands, ?Puerto Rico.

" " *pelioni* Comstock and Huntington. Hispaniola.

" " *burtiani* Comstock and Huntington. Florida.

" " *casasi* Comstock and Huntington. Cuba.

" " *armouri* (Clench). Bahamas.

" " *gossei* Comstock and Huntington. Jamaica.

Thecla martialis Herrich-Schäffer

Thecla martialis Herrich-Schäffer (1864) 164. Cuba.

Thecla martialis, Slosson (1901) 180.

Thecla martialis, Draudt (1920) 780, pl. 155, g.

We have seen this species from Florida, Nassau in the Bahamas, Cuba and Jamaica. The species is closely related to *acis*, as is obvious from its size, shape and pattern. The male genital armature is very similar in the two species.

Mrs. Annie Trumbull Slosson gave the food plant as *Trema micrantha* at Miami, Florida, and said: "larva a dull green with no markings, the whole upper surface covered with short bristle-like hairs of pure white."

Thecla favonius (Abbot and Smith)

Papilio favonius Abbot and Smith (1797) 1, 27, pl. 14. Georgia.

Thecla lipirops Boisduval and LeConte (1853) 90, pl. 31, figs. 1-4. Georgia.

Thecla favonius, Holland (1916) 495.

Thecla favonius, Draudt (1920) 798, pl. 158, e.

This species is common in Florida and occurs as far north as the Carolinas and along the Gulf coast. The supposed occurrence of this species in the Antilles is due to a misidentification made by Dr. Holland, who listed *favonius* from the Isle of Pines. This record was noted by Bates (1935: 193), who remarks, however, that he had seen no specimens from the West Indies. Dr. Avinoff located and examined the specimen (♀) on which Dr. Holland's record was based, and states in a letter: "It is a well-defined *Thecla angelia*."

The male genital armature is hardly to be distinguished from that of *acis*.

Godman and Salvin, in the *Biologia*, do not record any form of *acis*, *martialis* or *favonius* from Central America. These species seem to be predominantly an Antillean group.

Thecla angelia angelia Hewitson

Thecla hugo, Herrich-Schäffer (not Godart) (1862) 142. Cuba.

Thecla angelia Hewitson (1874) 162, No. 288 (part), pl. 63, fig. 139. Cuba.

Thecla angelia, Draudt (1920) 809, pl. 154, i.

This species was considered to be distinct from other Cuban species by Herrich-Schäffer, but he misidentified it under the name *hugo*. Hewitson recognized that the species was undescribed and gave the

name *angelia* to specimens he had from both Cuba and Jamaica, apparently not realizing that the two islands were inhabited by distinct populations. Hewitson, in describing the upperside of the male, gave the characters of the Jamaican population, but his description of the underside fits both sexes of the Cuban population and his first figure, No. 439, is a representation of a Cuban specimen. His description of the female fits the Jamaican population. We recognize the name *angelia* as applying to the Cuban population, because it is the first figured, and Cuba is the first locality mentioned. We recognize the Jamaican population as a distinct subspecies.

Draudt's figures of *angelia* are rather poor, but the upperside figure suggests the Jamaican subspecies and the underside figure might be of *angelia* from Cuba.

***Thecla angelia pantoni* NEW SUBSPECIES**

Thecla angelia Hewitson (1874) 162, No. 288 (part), pl. 63, fig. 440. Jamaica
Calycopis angelia, Kaye (1931) 535, pl. 39, fig. 12.

Size and shape: the length of forewing in both sexes is about 12 mm., the size and shape being the same as in *angelia*, except that the tail at Cu_1 is slightly shorter.

Ground color: this is dark brown on the upperside like that of *angelia*, but, on the underside of both sexes, it is brownish gray or "pale stone-colour," as stated by Hewitson for the male.

Markings: on the upperside the male has a fulvous area between M_2 and the inner margin, extending from the base three-quarters the length of the wing; the hindwing is slightly bronzed with fulvous scales (an effect overemphasized by Kaye's figure) and there is a spot of red at the anal lobe and sometimes a fulvous spot between the tails. The female has a slight bronzing over the whole ground color, as in the hindwing of the male, but lacks the fulvous patch of the forewing, being otherwise similar to the male. The underside (shown by Kaye's figure) is marked as in *angelia*, except that the mesial line of the forewing is curved basad at the costa, not straight; in the hindwing, the markings are generally fainter and the orange area is confined between the tails, with only faint traces of orange scales anterior to Cu_1 . The fringes are pale, somewhat tan in color (not brown as in *angelia*).

Types, all from Jamaica: holotype, male and allotype, female, Baron Hill, Trelawny Parish, 1150 feet, March 25, 1931, collected by Mr. E. L. Bell. Paratypes: same locality, ♂, ♀, March 16, 1931, ♀, March 25, 1931; ♂, Claremont, June 13, 1929; ♂, Claremont, June, 1929.

Paratypes from Hampton Court, ♂, July, 1937, and ♀, August 2, are in the collection of the Carnegie Museum, Pittsburgh, Pa.

Kaye recorded this subspecies as *angelia* from Jackson Town.

This subspecies is named for E. Stuart Panton, because of his excellent life history studies of the Lepidoptera of Jamaica.

This Jamaican subspecies is similar to *angelia dowi* (Clench) from the Bahamas, but differs in having the forewing cell area dark brown and the anal lobe red, not orange. On the underside of the forewing, the mesial line is curved basad at the costa and does not run straight to the costa, as in both *angelia* and *dowi*. Other small differences may be noted from the description of *dowi*.

Thecla angelia dowi (Clench)

Strymon angelia dowi Clench (1941) 4. Bahamas.

This subspecies, which was described from a series from several different islands of the Bahamas, is clearly distinct from *angelia* of Cuba. It is, on the other hand, very similar to *pantoni* from Jamaica, being differentiated only by minor but constant characters.

Thecla angelia boyeri NEW SUBSPECIES

Thecla endymion, Holland (not Fabricius) (1931) 242, pl. 64, fig. 32.

Size and shape: the length of forewing in males is 10 to 12 mm. and in females from 9.5 to 12 mm. The wings do not differ in size or shape from those of typical *angelia* of Cuba.

Ground color: this is the same dark brown as in *angelia* on the upperside in both sexes; on the underside, the males are distinctly darker brown than *angelia*, while the females are almost the same color as *angelia* but perhaps slightly more grayish than the ruddy brown to which *angelia* has a tendency.

Markings: in males, on the upperside, a fulvous patch extends from M_2 to the inner margin, beginning at the base and extending to about 1 mm. from the outer margin; the brown space at the outer border is slightly narrower than in *angelia* and the brown area anterior to M_2 is more densely blackish brown than in *angelia* (in which there is a tendency to a more extensive overscaling of fulvous); in the hindwing, the fulvous discal area is larger than in *angelia* for it almost reaches the tails (not being separated from the margin as in *angelia* by a broad, irregular brown space); the red-orange anal spot and the tails are as in *angelia*. The females are more variable than the males in the amount of fulvous on the upperside; some are bright, others are blackish brown with only traces of fulvous; generally, they are brighter and more fulvous than in *angelia*. The underside pattern, for the most part like that of *angelia*, differs in having all dark brown lines less sharply defined, in having the white spotting of the mesial line of the hindwing less sharply defined, in having the orange spots between Cu_2 and M_2

narrower and more clearly defined distad, and in having little tendency for orange scales to be present anterior to M_3 .

Types, all from Hispaniola: holotype, male, Pétionville, Haiti, May 20, 1930; allotype, female, San Lorenzo, Dominican Republic, June 24–26, 1931. Paratypes: 7 ♂, 6 ♀, Aux Cayes, Haiti, March 15–20, 1922; ♂, Pivert, Haiti, April 1, 1922; ♂, Port-au-Prince, Haiti, January 1–6, 1922; ♀, Punta Arena, San Lorenzo, Dominican Republic, June 24, 1915; 2 ♀, San Lorenzo, Dominican Republic, June 24–26, 27–29, 1915; ♂, ♀, Sanchez, Dominican Republic, May 28–31, June 3–6, 1915.

In the collection of the Carnegie Museum, Pittsburgh, Pa., there is one paratype ♂ labeled: "Hayti, Chipman," "Butterfly Book Pl. 64, Fig. 32." This is the specimen determined by Dr. Holland as *endymion* and figured in the Butterfly Book (1931). Through the courtesy of Dr. Avinoff, we have been able to examine this specimen and we determine it as *Thecla angelia boyeri*. The specimen is slightly atypical, in that the fulvous color invades the cell of the forewing more fully than is the case in the average specimen. The hindwing is also more broadly fulvous than usual, but the relative areas of fulvous and brown are variable in a series of specimens.

This subspecies is named for Jean Pierre Boyer (1776–1850), President of the Republic of Haiti.

This subspecies is readily separated from *dowi* and *pantoni* by the presence of fulvous color on the upperside of the hindwing, and the darker color and the more extended orange spotting of the underside. It also occurs in Puerto Rico as shown by a considerable series of both sexes, which do not differ appreciably from specimens from Hispaniola.

The subspecies of *angelia* are endemic in the Greater Antilles and appear in five areas sufficiently separated to maintain five distinct populations. Of these, the population of the Bahamas, *dowi*, and the population of Jamaica, *pantoni*, are very similar pale forms, so-called from the light underside coloring. Between these, there exists Cuban *angelia*, which is a dark form. In both Hispaniola and Puerto Rico, which probably maintain only slightly intermingling populations, there is another, even darker form, the subspecies *boyeri*, specimens of which from the two islands do not appear different. Why the populations of the Bahamas and Jamaica, two widely separated and differing environments, appear so much alike is a matter of conjecture.

Thecla pan (Drury)

Papilio pan Drury (1773) 2, 40, pl. 28, figs. 3, 4. Jamaica.

Thecla tirrhaca Moschler (1886) 14, Part 3, 26. Jamaica.

Thecla pan, Draudt (1921) 797, 824.

The striking black spot, rimmed basad with orange on the underside

of the hindwing, placed above Cu_2 , distinguishes this generally brown species which is known only from Jamaica.

Draudt's placing of *tirrhaea* Möschler as a synonym of *pan* Drury is apparently correct.

Thecla maesites maesites Herrich-Schäffer

Thecla maesites Herrich-Schäffer (1864) 165. Cuba.

Thecla maesites, Draudt (1920) 798.

We have seen this small species from Florida, Jamaica and Puerto Rico, and Clench (1941: 4) records it from the Bahamas. It is metallic, purplish blue on the upperside and green on the underside, except for a cinnamon and grayish marginal patch on the hindwing, which extends from the anal angle to about M_2 distad of the mesial line. This line is irregularly curved, not showing a "W" mark. Typical *maesites* has well-developed tails on the hindwing at Cu_1 and Cu_2 , the latter about twice the length of the former.

Thecla maesites clench NEW SUBSPECIES

PLATE 1, FIGURE 8

Size and shape: the length of forewing varies from 9.5 to 10.5 mm., the size being the same as in *maesites maesites*; the shape is also the same as that of *maesites*, except that, in both sexes, the tail at Cu_1 is absent and the tail at Cu_2 is only about 1 mm. long (in *maesites* the tail at Cu_1 is 1 mm. or more long and the tail at Cu_2 is 2.5 mm. long).

Ground color: the upperside of the males is metallic, purplish blue, decidedly duller than in *maesites*; the females are also of a darker and duller blue; the underside, alike in the sexes, is green, not differing appreciably from that of *maesites*.

Markings: the upperside of the males has an apical black area broadly concave basally, with a greatest width of about 2 mm., extending along the costa for about one-quarter of its length and extending along the outer margin to Cu_2 (this is not so in *maesites*); in females, the black costal and apical area of both wings is more extended and more intense than in *maesites*. On the underside of both sexes, in the forewing, the blackish area extends from M_2 to the inner margin, covering more than half of the wing (in *maesites* this area is more grayish black and extends from Cu_1 to the inner margin); in the hindwing, the marginal patch is more restricted than in *maesites* and the cinnamon as well as the blue and gray scales are less brilliant and the colors are more blended together; otherwise, the pattern is similar to that of *maesites*.

Types: holotype, male and allotype, female, and paratypes 2 ♂, 3 ♀, Roseau Valley, Dominica, B. W. I., April 11, 1929, collected by E. I. Huntington.

This subspecies is named for Mr. Harry K. Clench, in recognition of his interesting work on Antillean Lycaenidae.

* *Thecla maesites telea* Hewitson NEW COMBINATION

Thecla telea Hewitson (1868) 4, No. 10; (1871) 143, pl. 57, figs. 350, 351. Amazon

Thecla telea, Draudt (1920) 798, pl. 158, f.

Thecla telea, Holland (1931) 232, pl. 64, figs. 22, 23.

We have seen this subspecies from southern Brazil, British Guiana, Colombia, Mexico and Texas, and Draudt recorded it from Mexico to Paraguay. Holland considered it a Mexican species occasionally occurring in Texas and Arizona. It differs from *maesites* in having a much restricted brown patch on the underside of the hindwing, which stops just anterior to Cu₁, and in having the "W" mark of the mesial line of the hindwing well angulated. Photographs of the male type, made by Mr. C. F. dos Passos at the British Museum, confirm our determination.

Thecla crethona Hewitson

Thecla crethona Hewitson (1873) 157, No. 276, pl. 62, figs. 420, 421. Jamaica.

Thecla crethona, Draudt (1920) 799, pl. 158, f.

This distinctive species, metallic blue on the upperside and green on the underside, is larger than other Antillean *Thecla*, having a forewing length of about 17 mm. It is known only from Jamaica, whence we have seen a few specimens. It seems to be as closely related to *simaethis* (Drury) as to any described species.

Thecla simaethis simaethis (Drury)

PLATE 1, FIGURE 6

Papilio simaethis Drury (1770) 1, 3, pl. 1, fig. 8. "St. Christopher's."

Thecla sarita Skinner (1893) 112. Comal Co., Texas

Thecla lycus Skinner (1898) 48.

Thecla simaethis, Draudt (1920) 798, pl. 158, f.

Thecla simaethis, Wolcott (1936) 403.

The synonymy of *simaethis* is confused, because Skinner, while putting his own name, *sarita*, in synonymy (1898: 48), introduced a new name, *lycus*, attributing it to Hübner. *Urbanus Celebris lycus* Hübner (1807, Sammlung, 1: pl. [150]) is a large Castnid and cannot be the species Skinner had in mind. Possibly he meant *Bithys lydyus* Hübner (1818, Verzeichniss: 75, No. 753) which is a synonym of *Papilio eryx* Cramer (1777, 2: 75, pl. 143, D). Cramer's *eryx*, now placed in *Strymon*, is a species quite distinct from *simaethis*. Draudt apparently copied Skinner and perpetuated the error.

Drury figured a female *simaethis*, on the underside of the hindwing of which the outer margin is green, with a brown area next to the white mesial line. This is a reverse arrangement of the coloring as given in the description and in specimens. We have before us four male and

five female topotypes which agree with Drury's description and we think the name *simaethis* is correctly applied.

Thecla simaethis is widely distributed in the Antilles, being represented in the American Museum collection from Cuba, Hispaniola, Virgin Islands, St. Kitts, Dominica and St. Vincent. Wolcott recorded *simaethis* from Puerto Rico.

Thecla simaethis jago NEW SUBSPECIES

PLATE 1, FIGURE 7

Size and shape: the large size of this subspecies immediately makes it noticeable; the lengths of forewings in two males are respectively 15 mm. and 16 mm. and, in a series of 13 females, the smallest is 12 mm., several are 14 mm., and the average size is 13.2 mm. (These may be compared with *simaethis* from the type locality, St. Kitts, where the males measure from 11.5 to 13 mm. and the females from 11 to 12 mm. The other known populations are like those of St. Kitts or are slightly smaller in size. A series of *simaethis* from Dominica shows five males from 12 to 13 mm. and twenty females, the smallest 8.5 mm. and the largest 11 mm. with an average of about 10 mm.) In shape, the males of *jago* have a straighter outer margin of the forewing than typical *simaethis*. The forewing is also more apically pointed than topotypical *simaethis* and the hindwing is more elongate anally in the males. The females of the two subspecies do not differ in shape.

Ground color: this is brown on the upperside and green on the underside, not differing from typical *simaethis* in either sex.

Markings: the males, on the upperside, are glossed with purple, except for a narrow outer margin of brown; there is a streak of orange at the base of the costa of the forewing; the anal lobe is red; the females are brown with faint gray-blue overscaling spreading from the bases of the wings but otherwise similar to the males. The underside, in both sexes, is marked as in typical *simaethis*, except that, in the forewing, the gray-black area extends from just behind M_3 to the inner margin, covering more area than in *simaethis* (in which the green color often extends to Cu_1); in the hindwing, the difference in pattern is marked, for the whole mesial line is directed basad so that it meets the costa nearer the base, resulting in a widening of the green area at the costa, whence this is gradually narrowed down to a point at Cu_2 . (In *simaethis* the mesial line bends distad, so that, at the costa, it almost meets the brown marginal band and the included green area is much reduced.)

Types, all from Jamaica: holotype, male and allotype, female, Dunrobin District, Mandeville, Manchester, 2200 feet, January 7, 1920 and

November 28, 1919. Paratypes: ♂, ♀, Dunrobin District, Mandeville, Manchester, 2131 feet, December 23 and November 28; 2 ♀, Mandeville, Manchester, December 4-11; 2 ♀, Kendal District, Manchester, 1750 feet, January 19; 3 ♀, Constant Spring, St. Andrews, 650 feet, January 4-24, 25, 1920.

In the collection of the Carnegie Museum, Pittsburgh, Pa., there is one paratype ♀, Bull Head, Jamaica, August 17.

This subspecies is named for St. Jago, as Jamaica was first called by Columbus, who discovered it. This reference is from Moll's early 18th century map of Jamaica (Journ. Inst. of Jamaica, 1893, 1 (5): 185).

Thecla celida celida Lucas

Thecla celida Lucas (1857) 610. Cuba.

Thecla celida, Hewitson (1869) 125, pl. 49, figs. 246, 247. Cuba.

Thecla celida, Draudt (1920) 803, 159, b.

A half-dozen specimens of both sexes of this species from Cuba agree with Lucas' description and with Hewitson's figures made from a Cuban specimen. We consider the subspecies endemic in Cuba.

Thecla celida shoumatoffi NEW SUBSPECIES

Thecla celida, Kaye (not Lucas) (1931) 535, pl. 39, fig. 10. Jamaica.

Size and shape: these are the same as in *celida* from Cuba with a forewing length from 10.5 to 11.5 mm. The tails at Cu₁ and Cu₂ of the hindwing are less than half the length of those in *celida*.

Ground color: this is black at apex of the forewing and otherwise light metallic blue on the upperside, not differing from *celida*; on the underside, the color is dull grayish white (not bright blue-grayish white as in *celida*).

Markings: the male has a stigma like that of typical *celida* over the end of the forewing cell; in both sexes, on the upperside, in addition to the black apical half of the forewing, there is a narrow black marginal line on the hindwing with white fringes; on the underside, the linear pattern is similar to that of *celida*, but the lines are dull grayish black, less distinct than in *celida* and the mesial line on both wings is not white-bordered as in *celida*; there is a distinct, round, black spot ringed with white between the tails, and a small black spot at the anal angle (while in *celida* the spot between the tails is orange blending to yellow basad, and filling the space between the veins; also near the margin there are a few black scales in this spot and there are a few orange scales about the anal spot).

Types, all from Jamaica: holotype, male and allotype, female, Christiana, July 26, 1933, in the collection of The American Museum of Natural History. Paratypes: ♂, Christiana, July 26, 1933; ♂, Low River,

Trelawny, July 30, 1933; 2 ♀, Coleyville, Manchester, in the collection of the Carnegie Museum, Pittsburgh, Pa.; ♀, Monigue, St. Ann, July 20, 1933, in the collection of the Museum of Comparative Zoology, Cambridge, Mass.; ♂, Low River, Trelawny, July 28, 1933, in the collection of Cornell University, Ithaca, N. Y. All specimens were collected by A. Avinoff and N. Shoumatoff.

This subspecies is named for Mr. N. Shoumatoff, who captured many of the specimens.

Thecla celida aibonito NEW SUBSPECIES

Size and shape: these are the same as for *celida* from Cuba, except that the tails of the hindwing are short as in *shoumatoffi*.

Ground color: this is as in *celida* on the upperside, except that the apical black area is restricted and the metallic color is a deeper blue with a violet reflection; on the underside the white is not dull as in *shoumatoffi*, but has the same bluish tinge as *celida*.

Markings: the restriction of the black apical area is sufficient to allow the stigma at the end of the cell in males to stand out clearly on the blue ground (this is not so with either *celida* or *shoumatoffi* where the stigma is within the black apical area and thus obscured); on the underside, the tracery of the linear pattern is quite as in *celida*, but the black-pupiled orange spot between the tails is round, ringed with white (as in the black spot in *shoumatoffi*, and much smaller than in *celida*).

Types, all from Puerto Rico: holotype, male, Aibonito, July 14-17, 1914. Paratype: ♂, same data.

Thecla rufo-fusca Hewitson

Thecla rufo-fusca Hewitson (1877) 196, pl. 78, figs. 627, 628. No locality.

Thecla rufo-fusca, Godman and Salvin (1887) 2, 91. Guatemala, Pernambuco; (1896) 517. St. Vincent, B.W.I.

Hewitson described this species from a female. We know the species from St. Vincent and Central America.

Thecla angerona Godman and Salvin

Thecla angerona Godman and Salvin (1896) 516. St. Vincent, Grenadines, Grenada.

We recognize this species from topotypes from St. Vincent and a long series from Dominica. We have seen quite typical specimens from St. Kitts, from which island it was also recorded by Hall (1936: 277), which indicates that the species may have an extended distribution in the Lesser Antilles.

Thecla dominicana Lathy

Thecla dominicana Lathy (1904) 452. Dominica.

Thecla otokeba Dyar (1915) 423. Dominica.

Thecla dominicana, Draudt (1921) 808, 825.

Lathy described the male as *dominicana* and Dyar the female as

otoheba. A short series of both sexes from Dominica, taken at Cane-fields in December, flying at the same time and place with *angerona*, shows that the two species are distinct. Otherwise very similar to *angerona*, it may be distinguished easily by the presence of a red line on the underside of the hindwing which extends from Sc to M, basad of the mesial line.

The *columella* group

The following names, which are tabulated in their order of priority, with notation of sex and type locality, have been shuffled into various synonymic arrangements by different authors. There has been, heretofore, no satisfactory presentation of the names and the species and subspecies which they represent.

- 1780. *Papilio bubastus* Cramer, ♀, Cap de Bonne Espérance.
- 1793. *Hesperia columella* Fabricius, ♀?, Americae meridionalis Insulis.
- 1819. *Tmolus eurytulus* Hübner, ♂, ♀, no locality.
- 1866. *Thecla istapa* Reakirt, ♀, Vera Cruz, Mexico.
- 1868. *Thecla salona* Hewitson, ♂, Venezuela.
- 1868. *Thecla limenia* Hewitson, ♀, Jamaica.
- 1873. *Lycaena modesta* Maynard, ♀?, Florida.
- 1873. *Callicista ocellifera* Grote, ♂, Aurora, N. Y.
- 1874. *Thecla cybira* Hewitson, ♀, Cuba and Jamaica.
- 1874. *Thecla argona* Hewitson, ♂, Uruguay.
- 1902. *Thecla rana* Schaus, ♂, ♀, Castro, Paraná, Brazil.
- 1910. *Callicista laceyi* Barnes and McDunnough ♀, Del Rio, Texas.
- 1926. *Thecla eurytulus nigra* Lathy, ♂, Tucuman, Argentine.

Draudt (1920: 809) arranged the names as follows:

Thecla bubastus (Cramer); synonym *salona* Hewitson; tailless form.
Thecla bubastus eurytulus (Hübner); synonyms *cybira* Hewitson, *modesta* (Maynard), *ocellifera* (Grote), *istapa* Reakirt, *columella* (Fabricius); tailed form

Holland's (1931: 240) arrangement follows:

Thecla columella (Fabricius); synonyms *eurytulus* (Hübner), *cybira* Hewitson, *salona* Hewitson, *istapa*, Reakirt, *ocellifera* (Grote), *modesta* (Maynard), *laceyi* (Barnes and McDunnough).

Bates (1935: 194) gave the following arrangement for Cuba:

Strymon columella (Fabricius); synonyms *eurytulus* (Hübner), *limenia* Hewitson, *cybira* Hewitson. He further said: "In general, the names *eurytulus* and *cybira* have been used for females, *columella* and *limenia* for males, . . ." However, Hübner figured both sexes of *eurytulus*; Hewitson figured a female of *cybira*; Fabricius' description of *columella* is presumably of a female and Hewitson figured a female of *limenia*.

McDunnough (1938: 24) listed:

Strymon columella (Fabricius); synonyms *eurytulus* (Hübner); *istapa* Reakirt, *salona* Hewitson, *modesta* (Maynard), *ocellifera* (Grote).

The kindest comment is that these listings are slightly confusing, but we further suggest the perusal of Druce (1907: 627-628).

* *Thecla bubastus bubastus* (Cramer)

Papilio bubastus Cramer (1780) 4, 84, pl. 332 G, H. "Cap de bonne Espérance."

Thecla s. ilona Hesitson (1868) 31, No. 67, ♂, Venezuela, (1874) 139, pl. 63, figs. 429, 430, ♂. Amazon.

Thecla bubastus, Draudt (1920) 809, pl. 145, h, ♂.

This species is shown by the figure to have a length of forewing of about 15 mm. and a hindwing without a tail. Godart (1822: 638) said that this species came from the islands of America, not from the Cape of Good Hope. Other later authors have placed it in South America. The illustrations in different copies of Cramer's work are not uniform and some are quite poor, but a careful analysis of the two figures of the upper- and undersides of *bubastus* shows that they do represent the female of a butterfly occurring in Surinam and other parts of northern South America. Both sexes of this species have a gray-brown ground color on the underside, upon which the black spots, particularly basad in the hindwing, stand out clearly ringed with white. These spots seldom show any red scaling about them and the yellow crescent basad of the black spot anterior to Cu_2 is very narrow and pale in color. Some, but not all, specimens from Trinidad exhibit some red scaling about the black spots.

The size of Cramer's figure is a little large for South American specimens, which have a length of forewing of about 12 mm., but the wing shape is about correct, allowing for the fact that the produced anal angle is concealed by the way the figure is drawn. Superficially, Cramer's figure of the underside of *bubastus* resembles species of *Catochrysops* and associated genera from South Africa, but, upon critical examination, it is evident that the pattern of marking is quite different.

The pattern of the underside of *bubastus* is similar to that of *columella*, which is a species with tails on the hindwings, but diverges therefrom in the forewing because of the shifting distad of next to the last spot of the mesial row above Cu_1 . The last spot of the mesial row of the hindwing is angulate and not rounded as in *columella*, and it is bent basad toward the inner margin.

The male genitalia of *bubastus* were compared with those of *columella* and found to differ as follows: in *bubastus*, the aedeagus was slender-tipped and the saccus was relatively short; in *columella*, the aedeagus had a heavier tip and was about one-fifth longer, and the saccus was also relatively longer.

Bethune-Baker (1916: 452) gave some interesting observations on *bubastus* based on an examination of Cramer's original drawings in the British Museum. Bethune-Baker's description of the original drawing of *bubastus* leaves no doubt that this was of the South American species.

Thecla bubastus ponce NEW SUBSPECIES

Size and shape: these are the same as *bubastus*, but, in the type series, the males vary in length of forewing from 10.5 to 14 mm. and the females from 10 to 10.5 mm. The hindwings are tailless in both sexes and more rounded in females than in males.

Ground color: this is black-brown on the upperside and gray-brown on the underside, not differing appreciably from *bubastus*.

Markings: the macular pattern is the same as in *bubastus*, but it differs on the underside in that there is usually a certain amount of red scaling basad of the spots in the mesial rows in both wings and distad of the two basal spots in the hindwing. The round black spot above Cu_2 is usually prominent and it is capped with a broad crescent of color blending from orange to yellow as broad as the black spot. (The colored area in *bubastus* is narrowed to a line of pale yellow about one-third as broad as the black spot.) In males, the black spots in the anal area of the upperside of the hindwings are heavier than in *bubastus*.

Types, all from Puerto Rico: holotype, male, Ponce, July 20-22, 1914; allotype, female, Arecibo, July 30-August 1, 1914. Paratypes: 3 ♂, ♀, Arecibo, July 30-August 1; 2 ♂, "Aguarillo" (Aguadilla?), December; ♀, Caguas, May 28, 29; 2 ♂, ♀, Coamo Springs, December 26-29, July 17-19; ♂, Guayanilla, July 22; ♂, Manatí, June 27-29; ♀, Ponce, July 20-22; ♂, Quebradillas, January 2-3; ♀, San Juan, August 2-3.

Paratypes in the collection of Cornell University, Ithaca, N. Y.: 3 ♂, Aguacate (Aguadilla) April 25; ♀, Arecibo, July 30-August 1.

No subspecies of *bubastus* is recorded north of Puerto Rico, but populations of *ponce* appear in the Virgin Islands, St. Kitts, Antigua, Dominica, St. Lucia, St. Vincent and Grenada. These populations vary in small particulars, but all are distinct from typical *bubastus* of South America. The subspecies *bubastus* is represented in the American Museum collection by specimens from Surinam, British Guiana, Trinidad, Venezuela, Colombia, and Obidos, Brazil.

Thecla columella columella (Fabricius)

PLATE 1, FIGURE 13

Hesperia columella Fabricius (1795) 282, No. 83. "Americae meridionalis Insulis."
Tmolus columella, Butler (1869) 189.

We recognize *columella* as a tailed species which occurs in Hispaniola. This is an arbitrary selection of locality, since there is nothing in the original description to indicate which of several subspecies Fabricius had at hand. However, other species described by Fabricius

came from Hispaniola, so that he might have obtained *columella* from that island. This locality selection has the advantage of permitting the use of a recognized name for an additional subspecies.

Butler listed under *columella*, presumably as synonyms "L. Bubastus and Erytalus, Boisd. in Doubleday's List." In placing *bubastus* as a synonym of *columella*, he evidently followed the action of Godart (1822: 638). We are unable to find, prior to Butler's use of the name *erytalus*, any mention of this name in Doubleday's List (1847) or elsewhere. Our only recourse is to consider *erytalus* Butler a synonym of *columella*.

This butterfly has a forewing length of from 11 to 12 mm. in both sexes. The tails are about 1.7 mm. long in males and from 1.8 to 2.3 mm. long in females. On the underside of the forewing, in males, the spot in the mesial line above Cu_2 is usually slightly shifted basad and the delicate whitish scallops at the apex are usually well defined. In the hindwing, in both sexes, the light gray areas are obvious, with the round black spot at the margin between Cu_1 and Cu_2 capped with a very narrow edging of orange-red and, basad of that, an unsuffused light yellow crescent not over .5 mm. wide. In these respects *columella* from Hispaniola differs from other populations of the species.

* *Thecla columella modesta* (Maynard)

PLATE 1, FIGURE 12

Lycæna modesta Maynard (1878) 7, 178, ♀? Florida.

Calliope ocellifera Grote (1878) 178.

Thecla modesta, Morrison (1878) 188.

Thecla columella, Holland (1931) 240, pl. 64, figs. 35, 36.

This Floridian representative of *columella* is the largest of the subspecies with a forewing length of as much as 13 to 14 mm. The tails are long, being about 2.3 mm. in length in males, and 2.7 mm. in females. In females, the blue area of the upperside of the hindwing is more extended than in other subspecies, being well defined and bright to above M_3 and delicately suffused with blue to the costa in fresh specimens. At the apex of the underside of the forewing in males, the white scallops are obsolete to absent and the mesial line of spots is reduced. The black spot on the underside of the hindwing between Cu_1 and Cu_2 is prominent and it is capped with a large crescent of color which blends from red to light orange and is from two to three times as long as in *columella*. This is figured, but not overclearly, as *columella* by Holland. We have not seen the type of *ocellifera* (Grote), but we consider the synonymy probably correct.

Thecla columella cybira Hewitson

PLATE 1, FIGURE 15

Thecla cybira Hewitson (1874) 161, No. 286, pl. 63, figs. 435, 436, 9. Cuba and Jamaica.
Calloista bubastus eurytelus, Kaye (not Hubner) (1931) 535, pl. 39, fig. 13.

We consider this a slightly differentiated subspecies which occurs in Cuba and Jamaica. The size is about the same as in *columella*, but the tails are long like those of *modesta* in males, being from 2 to 2.3 mm., and, in females, from 2.2 to 2.4 mm. in length. In the females, the marginal spots on the upperside of the hindwings are more prominent than in *columella*, thus resembling the females of *modesta*, from which *cybira* differs by the absence of the extensive blue scaling. The underside ground color averages slightly darker in series of specimens. In males, the mesial line of spots on the underside of the forewing usually forms a curve and the posterior spot is not offset basad as in *columella*. The apical whitish scallops are usually obsolete. The marginal spot in the hindwing between Cu_1 and Cu_2 is usually capped with dark red-orange which extends basad about twice as far as in *columella*. This orange area resembles that of *modesta*, but is slightly deeper in color. The populations of Cuba and Jamaica are slightly different in small ways, as seen in comparative series, but we have not chosen to separate the Jamaican population by another name.

A short series of *columella* from several different islands of the Bahamas, loaned for study by Mr. Nathan Banks, is quite uniform in appearance. The size of individuals of this population is small, perhaps a little less in the average than that of *cybira*. The females show much blue color on the upperside of the hindwings, as in *modesta*. Both sexes are not only considerably smaller than *modesta*, but are less boldly marked on the underside. For the present, we consider the Bahaman populations as belonging to the subspecies *cybira* of Cuba and Jamaica.

Another short series, loaned by Dr. W. T. M. Forbes (1941: 147), came from the Dry Tortugas about the end of June, caught in sweeping, so that the condition of the specimens is far from good. This population is obviously related to *modesta* from Florida but seems slightly modified. A series of good specimens would be interesting to examine.

Thecla columella arecibo NEW SUBSPECIES

PLATE 1, FIGURE 14

Size and shape: the length of forewing in the males varies from 11 to 12.3 mm. and, in the female, it is 11 mm. The length of tails at Cu_2 in males is 1.5 to 1.8 mm. and, in the female, 2.2 mm. In the forewing, the outer margin makes a right angle with the inner margin so that the

shape or the forewing is that of a right angle triangle. The hindwing is produced and slightly lobed at the anal angle in the male and the whole wing more rounded in the female, but with a slight anal lobe.

Ground color: the color is dark gray-brown on the upperside of both sexes; on the underside, the color is light gray-brown.

Markings: the male has a circular stigma in the end of the cell (differing from other subspecies where the stigmas are elongate or slightly irregular); otherwise, both sexes have similar markings on both surfaces. On the upperside of the hindwing, there are rounded spots of darker brown near the margin on either side of Cu_2 and, in the female, there is a fainter spot anterior to M_3 . The fringes are white or smoky white. In the female, there is a circular area in the forewing of darker brown scaling surrounding the end of the cell. On the underside of the forewing, there are two marginal rows of faint brown-shaded spots, the inner much heavier, placed in the interspaces from Cu_2 to the apex. On either side of the inner row, there are traces of white scaling. Midway between the end of the cell and the margin, there is a row of darker brown, narrow spots outwardly banded with white, extending from Cu_2 to the costa and slightly convex distad, but well separated from the marginal spots at the lower end. In the hindwing, there are two black-brown, white-ringed spots below Sc , one basad, one distad. Midway of the cell, there is a small round brown spot scaled about with white and the end of the cell is closed with a brown bar with a few white scales about it. Beyond this bar, is a mesial row of brown spots, white-scaled distad, slightly red-scaled basad, starting below R_s and extending in an irregular, undulate arc to about midway of the inner margin. Between these mesial spots and the margin, there are two rows of brown spots darker than the ground color. These spots are placed in the interspaces, bordered basad with white scaling and are somewhat lunulate in form. Distad of these spots, there is a white and brown marginal line followed by a whitish fringe. The brown line extends so as to color the tail except for its white tip. The anal lobe is black, with red-orange scales basad; marginally in the interspace above $2A$ there is a black spot more or less overscaled with bluish white, and in the interspace above Cu_2 , there is a narrow black bar capped successively with red-orange and pale yellow (while in all other subspecies there is a prominent, usually circular, black spot which is more or less capped with color in various ways).

Types, all from Puerto Rico: holotype, male, Guayanilla, July 22, 1914; allotype, female, Arecibo, July 30–August 1, 1914. Paratype: ♂, Coamo Springs, January 6–10, 1915.

4 *Thecla columella istapa* Reakirt

PLATE 1, FIGURE 11

Thecla istapa Reakirt (1866) 330, No. 46, ♀ "Mexico, near Vera Cruz"
Strymon columella, Comstock & Dammers (1935) 120.

This subspecies occurs in Central America and ranges from Panama into Texas. It is the closest of the subspecies to typical *columella*, but the underside ground color is much paler and it is less distinctly marked, particularly in the hindwing pattern. It has a washed-out appearance compared with *columella* which, in turn, is less distinctly marked than *modesta*. The tails of *istapa* are the shortest in length of the known subspecies, being about 1.2 mm. long in males and from 1.5 to 1.7 mm. in females. Field (1940: 346) resurrected the name *istapa* for this subspecies of *columella*, making his comparison with Floridian *modesta*.

Thecla antigua NEW SPECIES

PLATE 1, FIGURE 16

Size and shape: the length of forewing is 11 mm. and the tail of the hindwing at Cu_2 is 2.5 mm.; the wingshape is like that of *columella* females.

Ground color: on the upperside it is black-brown and on the underside, gray-brown as in *columella*

Markings: on the upperside, this species has the same black spots on either side of Cu_2 , at the margin, as in *columella*; on the underside, the forewing has a double row of marginal spots, the outer one being barely discernible, the inner one faint, the mesial line is straight from the costa to Cu_2 (not curved as in *columella*); in the hindwing, the two basal spots are distinct and the cell bar is as in *columella*; the mesial line is made up of heavy blackish spots, each partly encircled with white, and these follow a more regular curve from the costa to the hind margin than in *columella*; counting from the costa, the second spot is directly below the first, not set basad as in *columella*, and thus the first four spots are in line, the fifth spot is separated and set but slightly basad, the small sixth and seventh spots slightly more basad and then, with a slight break, the eighth oblong spot extends at a right angle from the other spots toward the inner margin. The effect of this mesial line is striking, for it runs prominently across the wing in a way quite different from the mesial line in *columella*. The marginal brown spots are bold, with less white about them than in *columella*; the orange spot anterior to Cu_2 is relatively large with a marginal black bar; the anal black scaling is reduced with more white and orange scales about it

than in *columella*; in all these respects this species differs from *columella*.

Holotype, female, St. John, Antigua, B. W. I., June 5, 1911.

This species is definitely associated with *columella*. In addition to the type from Antigua, there is another specimen (♂, Tortola, V. I., March 29, 1925) which displays the same characters but of which we do not make a paratype. A comparison with the various subspecies of *columella* shows that *arecibo* from Puerto Rico is somewhat intermediate between *antigua* and typical *columella* from Hispaniola. We think that *antigua* may prove to be a subspecies of *columella* at the southern end of its island range, but, lacking intergrading material, we have described *antigua* as a species, thus calling attention to it, so that when more material from the Lesser Antilles comes to hand, its relationship may be correctly determined. Apparently *columella* forms are not widespread or common in the Lesser Antilles, for, other than the mention of "*eurytulus*" from St. Vincent and Grenada by Godman and Salvin (1896; 517), there are no records in the literature. We suggest that this record might have been a misidentification and that the species was *columella* or perhaps *bubastus*. *T. eurytulus*, so far as we know, is confined to continental South America.

Thecla toussainti NEW SPECIES

PLATE 1, FIGURE 17

Size and shape: the length of forewing in males is from 11 to 12.5 mm. and, in females, from 10 to 11 mm.; the tail at Cu_2 is about 2 mm. long in both sexes. The shape approximates *columella*, with which it flies at the same times and places, but the forewing is a little more apically acute and the hindwing a little more prolonged with the anal lobe more prominent.

Ground color: on the upperside, it is black-brown, slightly darker than *columella*; on the underside, gray-brown but appearing much lighter than *columella* because of the light gray overscaling.

Markings: on the upperside, the male has an obscure, round stigma at the end of the forewing cell; in the hindwing, there are two round black spots to either side of Cu_2 at the margin and a few orange scales at the anal lobe obscured by longer white, inner marginal hair-like scales; the hindwing fringes are white with brown scaling at the ends of the veins, giving a slightly scalloped appearance to the margin in the males; the females are like the males, except for a powdering of blue scales on the forewing from the base along the inner margin and blue scaling extending over the rear half of the hindwing. The general pat-

tern of the underside is like that of *columella*, but appears in more contrast because of the increased white scaling of the ground color. A definite difference in pattern appears in the forewing, where the third and fourth spots of the mesial row are set based out of line by their own width or more, thus interrupting the continuity of the line between M_1 and M_3 . In the hindwing, the mesial row of spots lacks the continuity of the row in *columella* and tends to an irregularity of placement of the spots and to obsolescence of spots. Nevertheless, the spot at the costa and the two basal spots are usually larger than in *columella* although variable in intensity, because, in some specimens, they are entirely black-brown and, in others, they have much red scaling. A peculiarity is the formation of the bar at the end of the cell by a streak of ground color surrounded by whitish (in *columella* there is a dark brown line). The black anal lobe and the blue scaled patch between it and Cu_2 are more prominent than in *columella*.

Types, all from Hispaniola: holotype, male and allotype, female, Fond Parisien, Haiti, February 11-18, 1922. Paratypes: 2 ♂, ♀; Fond Parisien, Haiti, February 11-18; ♂, Monserrat, Dominican Republic, July 20-22; 7 ♂, 7 ♀, Port-au-Prince, Haiti, January 1-6, 13, February 2-4, 19-28, March 5-11, April 6-8, 8-11, December 28.

Paratypes, ♂, ♀, from Haiti, Holland collection, are in the Carnegie Museum, Pittsburgh, Pa.

This species is named for Toussaint L'Ouverture, Liberator of Haiti.

Thecla christophei NEW SPECIES

PLATE 1, FIGURES 9, 10

Size and shape: the length of the forewing in the male is 12.5 mm. and, in females, from 12 to 13 mm.; the tail at Cu_2 is about 4 mm. long in both sexes and, in females, there is a slight vein projection at Cu_1 ; the anal lobe is well developed.

Ground color: on the upperside it is black-brown, as in *toussainti*; on the underside, it is pale grayish white, without indication of the gray-brown of *columella* (traces of which appear under the light gray over-scaling in *toussainti*).

Markings: there is an obscure stigma at the end of the forewing cell in the male; on the upperside, in the male, there are angular black spots on either side of Cu_2 at the margin of the hindwing, with some blue scales about them and the anal lobe is red scaled; in the females, there is a pale blue patch in the forewing, spreading from within the cell to the inner margin and two-thirds of the wing length distad; the hindwing is like that of the male, except that the pale blue area extends

from within the cell to the inner margin and distad to the outer margin along M_3 , and marginally as small spots to M_1 , thus making more than half the hindwing pale blue; the red anal lobe is well defined by contrast with the blue color. The underside pattern, alike in both sexes, is markedly different from either *columella* or *toussainti*; in the forewing, there is a double marginal row of linear spots and the mesial line has the third and fourth spots set out distad, more definitely in some specimens than in others, being the reverse of the arrangement in *toussainti*; in the hindwing, the two marginal rows of gray-brown spots are better defined than in *columella* or *toussainti*; the black marginal spot above Cu_2 is larger and capped basad by a large pale yellow spot, filling the space between veins; the dark mesial line is discontinuous, made up of a large, costal spot, paired spots beyond the end of the cell, a spot above Cu_2 , another below, which is semicircular, and a bar from 2A to the inner margin; these spots are lightly edged distad with white scales, and the anterior three inwardly with red, which is defined basad with a line of dark scales; the cell bar is not always distinct and, within the cell, there may be present a circular spot, usually obsolete.

Types, all from Hispaniola: holotype, male, Port-au-Prince, Haiti, January 1-6, 1922; allotype, female, Paradis, Dominican Republic, 1800 feet, August 15, 1932. Paratypes: ♀, Port-au-Prince, Haiti, January 1-6, 1922; ♀, Pétionville, Haiti, June 13, 1930; ♀, San Lorenzo, Dominican Republic, June 27-29, 1915.

This distinct species flies with *columella* and *toussainti*, at the same times and places in Hispaniola. For purposes of description, it has been compared with them, but, if the hindwing is compared with *limenia*, there is a distinct resemblance both in shape and underside pattern and we have the feeling that this new species has more fundamental characters in common with *limenia* than with *columella*.

This species is named for Henri Christophe, onetime ruler of Haiti.

This might possibly be the species misidentified as *cardus* Hewitson by Dewitz (1877: 241) in his list of species from Puerto Rico. If so, the species may be expected from Puerto Rico, but it has not appeared in any recent collections.

Thecla limenia Hewitson

Thecla limenia Hewitson (1868) 92, No. 69, ♀, Jamaica; (1874) 160, pl. 68, figs. 431, 432, ♀. Jamaica, St. Domingo, Cuba.

Thecla limenia Draudt (1920) 809, pl. 145, i.

Callioida columella, Kaye (not Fabricius) (1931) pl. 59, fig. 15.

We recognize this as an entirely distinct species, endemic in the Antilles, which is recognizably figured by Draudt. It is represented in the collections by many specimens of both sexes from Cuba, Jamaica,

Hispaniola, and Puerto Rico. This species is slightly larger than *columella*, but of quite similar appearance on the upperside in both sexes, except for the presence of traces of red scaling in the anal area of the hindwings. The underside is very different, although the distribution of the maculation is the same. The ground color is much browner than in any subspecies of *columella*. The mesial line of spots of the forewing is nearly straight and is more nearly parallel with the margin. This line in the hindwing is more definite, and at 2A it bends at a right angle toward the inner margin. The two basal spots and the costal spot of the mesial line are large, round and clearly circled with white. The orange spot above Cu_2 is emphasized to about four times the length of that spot in *columella*.

• *Thecla eurytulus eurytulus* (Hübner)

Tmolus eurytulus Hubner (1819) Sammlung, 2, pl. 90, figs. 1-4, ♂, ♀. No locality.

Thecla argona Hewitson (1874) 162, No. 289, pl. 63, figs. 441, 442, ♂. Uruguay.

Thecla rana Schaus (1902) 414. Castro. Parana, Brazil.

Thecla eurytulus, Lathy (1926) 46.

We recognize *eurytulus* as a species which occurs at Santa Catharina, Brazil. Lathy expressed the opinion that *argona* was a synonym of *eurytulus* and this may well be so. On the authority of Druce (1907: 628), *rana* may also be placed in synonymy. The figures of Draudt (1920: pl. 145, h) show a *columella* form labelled *eurytulus* and actual *eurytulus* (tails missing) labeled *argona*.

Among the very extensive Antillean collections, we do not recognize any form of *eurytulus*.

Compared with *columella*, *eurytulus* is similar on the upperside except that in the marginal area of the hindwing of *eurytulus* there is a row of black spots extending from the anal angle through two to four interspaces; these spots are more or less encircled with blue, which usually does not suffuse over the wing. There is more or less red scaling at the anal lobe and sometimes beyond, about the black spots. The underside of *eurytulus* is decidedly different from that of *columella*. The mesial row of spots across both wings of *eurytulus* is made up of definitely separated spots, and in the hindwing there is no definite bar-like spot closing the cell as in *columella*. The black pupil above Cu_2 is minute and set in a round red spot. The anal lobe has red scaling and there is often considerable red scaling along the bands of spots. On the underside, the males of *eurytulus* are duller and darker than *columella* and the females brighter. In neither sex, is there much evidence of the grayish white submarginal markings present in *columella*.

* *Thecla eurytulus nigra* Lathy

Thecla eurytulus nigra Lathy (1926) 46, ♂. Tucuman, Argentina, 9.VI.1922.

The subspecies *eurytulus nigra* from western Argentina is darker

than typical *eurytulus* on the underside, having a smoky appearance with the marking less definite. We also recognize this subspecies in specimens from Chile.

A summary of the *columella* group indicates the species and subspecies with their synonymy as we recognize them.

- Thecla bubastus* *bubastus* Cramer). Surinam (Cap de Bonne Espérance).
 " synonym *salona* Hewitson. Venezuela.
 " *ponce* Comstock and Huntington. Puerto Rico.
columella *columella* (Fabricius). Hispaniola (Am. mer. Insulis).
 " *cybira* Hewitson. Cuba, Jamaica.
 " *arecibo* Comstock and Huntington. Puerto Rico.
 " *modesta* (Maynard). Florida.
 " synonym *ocellifera* (Grote). Aurora, N. Y.
 " *istapa* Reakirt. Vera Cruz, Mexico.
antigua Comstock and Huntington. Antigua.
toussainti Comstock and Huntington. Hispaniola.
christophe Comstock and Huntington. Hispaniola.
limenia Hewitson. Jamaica.
eurytulus *eurytulus* (Hübner). Southeastern South America (no locality)
 " synonym *argona* Hewitson. Uruguay.
 " synonym *rana* Schaus. Castro, Paraná, Brazil.
 " *nigra* Lathy. Southwestern South America (Tucuman, Argentine).

Callicista laceyi Barnes and McDunnough (1910: 365, ♀. Del Rio, Texas) was very positively placed in the synonymy of *columella* by Holland (1931: 240). From a careful reading of the description and examination of photographs of the type, we place *laceyi* as a good species which is closely related to *clytie* Edwards. We do not consider it as a member of the *columella* group.

* *Thecla bazochii bazochii* (Godart)

- Polyommatus bazochii* Godart (1822) 681, No. 203. Brazil.
Eureus thius Geyer (1852) Zutrage 4, 38, figs. 743, 744. Brazil.
Thecla agra Hewitson (1871) 147, pl. 58, figs. 369, 370. Amazon.
Thecla thius, Druce (1907) 630.
Thecla thius, Draudt (1920) 810, pl. 145, i, k.

Godart's description rather clearly defines this small species which we know from numerous localities in eastern and northern South America, Central America and Mexico. We consider our synonymy correct, which accords with that of Draudt, except that *bazochii* has priority, and we hesitate to include *infrequens* Weeks (1901: 265; 1905: 37, pl. 6, fig. 3. Cusilluni, Bolivia). We have no Bolivian or western coast specimens and Weeks' figure certainly suggests a somewhat different butterfly. Lathy (1926: 47) said "The type of *bazochii*, Godt. is in the Paris Museum, and it is undoubtedly the same species as *thius*." Considering the amount of variable suffusion in the markings of the underside of this species in any series, there is no difficulty in reconciling the various descriptions and figures.

Thecla bazochii gundlachianus (Bates) NEW COMBINATION*Strymon gundlachianus* Bates (1935) 195. Cuba.

This subspecies appears in various similar populations in Cuba, Hispaniola and Jamaica with no apparent differentiation between the islands. It may be separated from continental *bazochii* by the less distinctive pattern of the underside of the hindwings, where there is a generally mottled brown suffusion with considerable reduction of the white markings.

The male genitalia of Cuban *gundlachianus* and *bazochii*, from the region of Pará, Brazil, were compared and found to be the same. We therefore place *gundlachianus* as a subspecies of *bazochii*.

Plebejinae

We use this subfamily in the currently accepted sense, to separate the following species from the Theclinae, but the division is not clearly defined.

LEPTOTES Scudder

Leptotes Scudder (1876) 124.Genotype.—*Lycæna theon* Lucas, 1857.The *cassius* group

Leptotes cassius has a very wide range, extending southward from Mexico on the continent and from Florida through the Antilles to Argentina and Chile. It appears in many subspecific populations, both on the continent and on the islands, but we will discuss principally the island populations.

From a study of the extensive material available, it is our opinion that *cassius* is a species of South American origin, usually inhabiting lowlands and relatively moist environments. It is divided into two main groups, the continental and the insular, each having distinctive characters which hold throughout the two groups of associated subspecies. From the evidence we have, we believe *marina* Reakirt to be a related allopatric species of entirely continental distribution which does not enter into this discussion.

* *Leptotes cassius cassius* (Cramer)

PLATE 1, FIGURE 21

Papilio cassius Cramer (1775) 1, 36, pl. 28, C. D. Surinam.*Polymmatius cassius*, Godart (1823) 679, No. 192.*Hemiargus (Leptotes) cassius*, d'Almeida (1933) 230. Life history.

Cramer figured both sides of a Surinam female fairly accurately. Specimens from the Guianas and from Trinidad were compared with Cramer's figures and found to match excellently; they have extensive pure white areas on the upperside, with the dark brown markings

sharply defined; the shining blue is closely confined to the wing bases, as specified by Cramer's text; the underside pattern agrees well. The males also agree with Cramer's underside figure. On the upperside, the males have white hindwings with a violet-blue margin 2 mm. or less wide and, in the majority of the specimens, the forewings have a white semicircular area extending from the inner margin to the cell, with the remainder of the wings violet-blue. Godart first recognized and described the male, giving Brazil as well as Surinam for the localities.

Continental *cassius* of Surinam extends southeasterly to Argentina and reaches the affluents of the Amazon in Peru, exhibiting some variation. In Venezuela and Colombia, it is nearly typical, but specimens from Panama show a change which holds for the slightly intervarying populations extending northward even into southern Texas. These Central American populations we will now consider as one subspecies, recognized as *striata* Edwards, which has developed characters which resemble the Antillean populations. We consider that Barnes and McDunnough (1916: 107) improperly placed *striata* in the synonymy of *floridensis* Morrison, a practice followed by later authors.

* *Leptotes cassius striata* (Edwards)

Lycaena striata Edwards (1877) 86. San Antonio, Texas.
Lycaena cassius, Godman and Salvin (1887) 2, 105.

Described from specimens from the northern end of the range, this name may be used in a broad way for all of the North and Central American populations of *cassius*, specimens of which we have seen from as far south as Panama. On the upperside, *striata* has only a little white scaling in the males, so that they are predominantly violet-blue, and the females have the dark brown markings increased with the blue coloring extended from the base, so that the white areas are greatly reduced. On the underside of both sexes, there is less white and more brown, so that the pattern is heavier than in *cassius*, but the brown is definitely paler. In the hindwing, the two marginal eyespots are larger than in *cassius* and round, not marginally elongate. Central American *striata* is definitely nearer the insular group than is South American *cassius*.

Godman and Salvin used the name *cassius* in an all-inclusive sense for all continental and insular populations.

We lack specimens from the southern islands of the Lesser Antilles, but make note of one poor specimen from Martinique, a male, which has much white in the hindwings and is essentially of the continental type on the underside.

In Dominica, B. W. I., there is a great change in the population, and

thence, northward, all specimens from many islands are of what we call the insular type, which carries into southern Florida. Either sex of any of the island populations can be separated at a glance from continental populations by the larger size and greater prominence of the marginal eyespots on the underside of the hindwing.

We will now consider the first subspecies of the insular group to be described.

Leptotes cassius catilina (Fabricius) NEW COMBINATION

PLATE 1, FIGURES 29, 30

Heesperia catilina Fabricius (1793) 3, 304, No. 150. "Americae meridionalis Insulis."
Lampides catilina, Butler (1869) 165. Jamaica?

Fabricius located his species as from the islands. A survey of various island populations showed that the subspecies of *cassius* occurring, with considerable uniformity, in the various Virgin Islands best agreed with his description. A series from St. Thomas agrees in every particular and this is not surprising, for Fabricius is known to have received other species from this island and this may have been the source of his type of *catilina*. Fabricius compared *catilina* with his *damoetes*, now regarded as a synonym of *baeticus* Linnaeus, which is a rather apt comparison. The type was obviously a female, of which he says: "Alae omnes supra fuscae, coerulesco nitidae. Subtus pallidiores fasciis plurimis, albis. Angulus ani ocellis duobus dimidio coeruleis atrisque." The females from the Virgin Islands usually have "All wings above fuscus, blue shining." However, some specimens show discal white scaling. On the underside, the brown is "paler" than on the upperside "with very many white lines." In the hindwing, "the anal angle with two ocelli half blue and half black" fits the specimens, for these ocelli are very distinct, blue and black with a very little ochre coloring about them in some specimens.

Butler determined something as *catilina* occurring questionably in Jamaica. From his remark "subtus albo fasciatis" it would seem that he had a butterfly like *cassius*. It seems quite probable that he determined some one of the insular populations of *cassius*, perhaps the correct one, but he was uncertain of the locality.

We can not agree with Bethune-Baker (1916: 454) in placing *catilina* as a relative of *ammon* Lucas from Arizona and Florida or from Haiti and St. Domingo. It is quite impossible to fit "Subtus pallidiores fasciis plurimis, albis" to *ammon* or any relative thereof. His determination, unfortunately, has been quite generally accepted, with the result that the name *catilina* has been applied to the subspecies of *ammon* occurring in Florida. We believe this to be entirely incorrect.

Bethune-Baker said that Holland's figures labeled *ammon* were *catilina* (1898: 270, pl. 30, fig. 45; pl. 31, fig. 31). This can not be so, for Holland's figures, in no way agreeing with the Fabrician description, are recognizable figures of the Floridian subspecies of *ammon*. In the revised edition of The Butterfly Book (1931), Holland accepted Bethune-Baker's determination of his figure as *catilina* and so renamed them.

The males of *catilina* are of a much deeper violet-blue on the upperside than the males of *cassius* and lack entirely the white scaled areas. The underside is heavily marked with brown as in the females, and both sexes are much darker than the sexes of *cassius*, but it is obviously a related subspecies.

The populations of the Virgin Islands which we determine as *catilina* are the darkest of any of the island races known to us, but we consider the population of St. Kitts properly grouped with them, though showing a tendency toward another population in the Lesser Antilles.

The second name proposed for an insular population of the *cassius* group is *theonus* from Cuba. Although the populations of each island of the Greater Antilles are, perhaps, minutely different, we apply the name *theonus* broadly to specimens from the Bahamas, Cuba and Jamaica. The populations of Hispaniola and Puerto Rico are also tentatively included, although these seem to form a transition between *catilina* of the Virgin Islands and *theonus*. The Floridian population of *cassius* was described by Morrison as *floridensis*, but we elect to place this name in the synonymy of *cassius theonus*, because it appears to us even less differentiated from some of the various island populations, placed broadly as *theonus*, than these various populations are separated from each other.

Leptotes cassius theonus (Lucas)

PLATE 1. FIGURES 26, 27

Lycæna theonus Lucas (1837) 611, pl. 16, figs. 8, 8 a, b. Cuba.
Lycæna cassius var. *floridensis* Morrison (1873) 187. Florida.
Leptotes theonus, Bates (1885) 108.
Leptotes cassius theonus, Clench (1942) 243.

The males of typical Cuban *theonus* have a slightly paler violet-blue color on the upperside than has *catilina*, and there is more or less white scaling along the anal fold of the hindwing. The underside pattern resembles that of *catilina*, but the brown markings are reduced and tend to obsolescence toward the outer margin of the hindwing. The females, on the upperside, are not so dark and smoky as is *catilina* and have much clearer and larger white areas with a blue suffusion over the forewing, which reaches the broad, black-brown marginal band with the separation clearly defined. The two black marginal eyespots

of the hindwing are distinct, the outer one the larger and the inner usually of less than half the diameter, with a ring of blue scaling within each spot which is sometimes surrounded by an ochre ring varying in intensity in individual specimens. (In *catilina*, the eyespots are more nearly equal in size with the smaller, being at least half the diameter of the larger one.)

Clench placed *floridensis* as a synonym of *theonus* and placed *theonus* as a subspecies of *cassius*. His inclusion of Mexican and Honduran specimens under *theonus* does not agree with our findings.

Haskin (1933: 154) gave life-history notes on *theonus*.

We will now consider a subspecies of *cassius* which appears in Dominica and Guadeloupe, B. W. I.

Leptotes cassius chadwicki NEW SUBSPECIES

PLATE 1, FIGURE 28

Syntarucus cassius, Butler (1901) 712.

Size and shape: the males have a length of forewing from 9.5 to 12.5 mm. with an average of 11 mm. for six specimens, and the females from 9.5 to 13 mm. with an average of 11.5 mm. for fifteen specimens. The size, like that of other insular subspecies, is smaller than that of *cassius cassius*; the shape is typical.

Ground color: in males, the upperside is dark violet-blue and the underside white as in *catilina*; the females, on the upperside, might be said to have a white ground, much obscured by the pattern, but the underside is like that of the males.

Markings: the male is without marking on the upperside, with a thin marginal black line and with fringes inwardly brown, outwardly white, more extensively white on the hindwing; the female, on the upperside of the forewing, has a costal and outer marginal black-brown border, increasing from about 1 mm. at the base, extending toward the apex and then to the tornus with its greatest width, measured along Cu_2 , of 2.3 mm.; the white disk of the wing may be completely suffused with bright blue, or only so in the basal third; the heavy macular pattern, placed as on the underside, is represented on the upperside by a number of black-brown, sometimes blue-suffused spots; the hindwing has the costal edge broadly black-brown, while the markings of the outer margin are of variable intensity; the white ground may be more or less suffused with bright blue, the veins are lined with brown, and brown spots may be present or absent in and at the end of the cell; a marginal black spot seems to be consistently present anterior to Cu_2 and usually there is a smaller one behind Cu_2 .

The underside pattern is alike in the sexes. There is the usual brown design of *cassius* on a white ground with marginal eyespots on the hindwing, but the pattern has undergone considerable modification. To explain the change, largely one of omission of marking, *chadwicki* may be compared with *catilina*. As a basis of the pattern in the forewing, in all subspecies of *cassius*, there is a prominent central solid brown line or a line of spots from the costa to the inner margin, and a second line of spots distad, beginning at the costa and ending at Cu_1 . The intensity of these lines is variable in any population, but in *catilina* and *chadwicki* the lines are heavier than in any others of the subspecies of *cassius* and the second, shorter line tends to a distinct curvature, bulging toward the apex. Extending from the costa, between and to either side of these main lines, there are more or less lighter lines which occur in all subspecies of *cassius* except *chadwicki*. In *chadwicki*, these three secondary lines are obsolete to absent, with the result that the pattern is strikingly different from all other subspecies of *cassius*, but most closely following the pattern of *catilina*. The brown hindwing pattern of *chadwicki* is similar to, though lighter than that in *catilina*. The eyespots are very distinctive, for, in *chadwicki*, these are larger than in any of the other subspecies of *cassius*, being elliptically elongate, not round; the anal spot is but little smaller in size than the one beyond Cu_2 ; both black spots are clearly ringed with metallic blue scales within, and with ochre exteriorly.

Types, all from Dominica, B. W. I.: holotype, male and allotype, female, Roseau Valley, April 11, 1929. Paratypes: ♂, 3 ♀, Roseau Valley, April 11, 1929, collector E. I. Huntington; 3 ♂, 8 ♀, Cane-fields, November 12 to December 8, 1933, collector L. E. Chadwick; ♀, Mt. Joy, 1400 feet, November 9, 1933; ♂, ♀, Dominica, September 19.

There are also from Guadeloupe: ♂, ♀, Basse Terre, March 17, 1929; ♀, St. Claude, February 21, 1941, collector A. Blanchard. These specimens are recognized as *chadwicki* but are not made paratypes.

This subspecies is named for Mr. L. E. Chadwick, who collected many of the specimens.

Butler said of specimens of *cassius* from St. Lucia and Dominica that he had not seen any continental examples so boldly marked or with so few bands on the under surface of the primaries.

* *Leptotes marina* (Reakirt)

Lycena marina Reakirt (1868) 87. Mexico.

Both sexes were described with the localities given as Orizaba, Mex-

ico, and "Mexico, near Vera Cruz." Reakirt remarked "Allied to *Lycaena cassius*, Cram." As we know *marina*, it is distributed in the western regions of the continental area from Chile to California. It occurs in the Galapagos Islands but we have never seen it from the West Indies.

Wolcott (1936: 402) gave a record as follows: "*Lycaena marina* Reakirt-det. W. Schaus at Camuy (EGS)." We have not seen this specimen, which may have been *marina* casually introduced in Puerto Rico, or, as is likely, it may have been a specimen of *cassius theonus*, which is often dark and heavily marked in Puerto Rico, approaching *cassius catilina* occurring in the Virgin Islands.

Leptotes perkinsae Kaye

Leptotes perkinsae Kaye (1931) 534, pl. 39, figs. 2, 5. Jamaica.

This species is very distinct, being well figured by Kaye in both sexes. We have seen males from Baron Hill, Trelawny, and Claremont, taken in March, June and July, and from Montego Bay, in November.

The occurrence of *perkinsae* in Jamaica flying, together with *theonus*, is very interesting.

HEMIARGUS Hübner

Hemiargus Hübner (1818) Zutrage, 1, 19, No. 50.

Hemiargus Hübner (1819) Verzeichniss, 69.

Hemiargus, Hemming (1934) 104.

Genotype.—*Hemiargus antibubastus* Hübner, 1818 (= *Hemiargus hanno antibubastus* Hübner).

The *ammon* group

The distribution, as it is known to us, of the species *Hemiargus ammon* (Lucas), is the southern half of Florida, the Bahamas, Cuba, Hispaniola, Puerto Rico and the Virgin Islands as far as Anegada. Its occurrence elsewhere has been recorded as Arizona by Bethune-Baker and Yucatan by Lucas. We have seen much material from both of these last two localities, but in it we have never seen *ammon* in any of its subspecies. We are inclined to doubt the correctness of these records. Hoffmann (1940), in his rather complete and carefully compiled list of species of the Mexican region, gave no record of *ammon*.

We will consider the subspecies of *ammon* in the order of their description.

Hemiargus ammon ammon (Lucas)

PLATE 1, FIGURE 22

Lycaena ammon Lucas (1857) 612, pl. 16, figs. 7 ♂, 7 ♀, a, b ♀. Cuba.

Lycaena filenus, Holland (not Poey) (1931) 270, pl. 68, figs. 2 ♂, 3 ♀, 4 ♂ underside.

Hemiargus ammon, Bates (1885) 197.

The specimens described by Lucas came from the neighborhood of Havana. We believe, from his figures, that he had a winter male and

a summer female, for there are seasonal differences appearing in both sexes.

A winter brood male was the first described and the name *ammon* is definitely assigned to that brood. In it, the upperside blue is a little brighter and the black-brown marginal line, particularly in the forewing, is a little more intense than in the summer male. In the hindwing, the second marginal black spot in front of Cu_1 has but the faintest trace of pink basad of it. On the underside, no seasonal differences are noted. In summer males, the color is more violet-blue and thinner, so that the underside pattern shows through. The black spot above Cu_1 has a definite pinkish patch basad of it.

The winter brood female has much more blue on the upperside of both wings, the black-brown margins being about 2 mm. wide. In the summer brood female, the black-brown areas usually cover more than the distal half of the wings. The red-orange patch over the black spot, in front of Cu_1 , may extend 2 mm. basad, being over twice the size of that patch in the winter brood. As in the male, no seasonal differences are noted on the underside.

The distinguishing characters of typical *ammon* are the considerable whiteness of the underside and the nature of the basal spots of the underside of the hindwing. There are usually only three black-centered basal spots, for the basal spot placed between Cu_1 and Cu_2 is usually absent and, if present, it is obsolete and not black-centered. These characters separate Cuban *ammon* from the other insular subspecies.

We do not choose to define the summer form of *ammon* by a different name, because it would serve no useful purpose.

Gundlach (1881: 72) gave the food plant as "Brasilete (*Caesalpinia*)."

Clench (1943: 57) doubtfully recorded typical *ammon* from Long Island in the Bahamas. We question his statement that *ammon ammon* occurs Hispaniola.

Holland's use of the name *filenus* and his figures, as cited, require special comment. He described and figured females of Cuban *ammon*, taken at Nueva Gerona, Isle of Pines. Holland said that the butterfly he defined "has been found on the Indian River and elsewhere in southern Florida." We have before us an extensive series of the Floridian representative of *ammon*, from the Indian River and many other places in southern Florida, taken at all seasons of the year, but all are definitely different from Cuban *ammon*. We doubt the occurrence of typical *ammon* in Florida, except possibly as a casual visitor. Holland's misidentification of *filenus* (Poey), which is related to *hanno* (Stoll), is

hard to explain when we think of the splendid figures of Poey, which must have been known to Holland.

Hemiargus ammon thomasi Clench NEW COMBINATION

Hemiargus catilina thomasi Clench (1941a) 407. Bahamas.

The author said that this subspecies differed from the Floridian population "chiefly in the reduction and graying over of the white bands on the under surfaces . . . It is even more distinct from the Cuban race, *ammon*, since that form is very light, and has very broad, white bands below." The types came from Arthurs Town and Russell's Creek, Cat Island.

We have before us a male from Nassau, New Providence Island, February 6, which agrees with specimens of the summer brood of *ammon* in having a pink spot basad of the black spot between Cu_1 and Cu_2 on the upperside of the hindwing. The underside is hardly darker than in *ammon*, showing as much white as some males. A female from New Providence Island, November 14, is quite inseparable, on the upperside, from winter specimens from Florida, but, on the underside, the amount of white marking is definitely reduced as compared with usual specimens from Florida.

Considering the wide separation of many of the Bahama islands, a considerable diversity among the populations in different islands may be expected.

Clench described *thomasi* as a subspecies of *catilina*, following the currently accepted determination of the latter name by Bethune-Baker. We consider this an error, as previously pointed out, and we are therefore proposing a new name for the Floridian population.

Hemiargus ammon bethune-bakeri NEW SUBSPECIES

PLATE 1, FIGURE 25

Lycaena ammon, Holland (not Lucas) (1898) 270, pl. 30, fig. 45, pl. 31, fig. 31, ♀.

Hemiargus catilina, Bethune-Baker (not Fabricius) (1916) 454.

Hemiargus ammon, Grossbeck (not Lucas) (1917) 83.

Lycaena catilina, Holland (not Fabricius) (1891) 270, pl. 30, fig. 45, pl. 31, fig. 31, ♀.

Size and shape: this subspecies is of the same shape, but of definitely larger size than Cuban *ammon*. An average taken for ten males gave a length of forewing of 11.8 mm. and a similar average for females of 11.9 mm.

Ground color: the male is blue on the upperside, slightly less violet and duller than *ammon*; in the female, there is a similar change of color; on the underside, both sexes are gray-brown, less tinted with white than in *ammon*.

Markings: in the male, on the upperside, the margins are more broadly black-brown and the two marginal spots at Cu_1 and Cu_2 are

less prominent than in *ammon* and, in a large series, do not show the pink tinting basad of the black spot anterior to Cu_1 (present in both broods of *ammon*); in the winter brood male, the two marginal black spots of the hindwing are reduced, with the anal one sometimes obsolete or even absent. In the female, the winter brood has narrow black-brown borders and much blue as in *ammon*; the colored lunule basad of the black spot at Cu_2 is orange instead of red-orange, as in *ammon*, and it is less produced basad.

The underside of both sexes is similar, and differs from *ammon* in that there is less white tinting in the basal and in the marginal areas of both wings, so that broad bands of white (2 mm. wide more or less) appear across both wings, these being cut only slightly by the brown scaling along the veins. In the forewing, the postdiscal white band (instead of being narrowed posteriorly as in *ammon*) is broad throughout. In the hindwing, the postdiscal white band is relatively broader than in *ammon* and the pale orange spot behind Cu_1 is reduced to a narrow crescent (in *ammon* this spot is red-orange and produced basad with a length equal to the width of the white band).

Types, all from southern Florida and of the winter brood: holotype, male and allotype, female. Miami, January 26, 1932. Paratypes: 2 ♂, 2 ♀, Miami, January 26, 1932; ♂, ♀, Miami, February 12, 1932; ♂, ♀, Royal Palm Park, April 12-18, 1923; 2 ♂, Royal Palm Park, March 23-24, 1929; 2 ♂, Key Largo, February 18, 1932, April 9, 1938; ♀, Punta Rossa, April 3, 1912; 5 ♂, ♀, Port Sewall, February 14, 1938, November 16-18, 1938, January 4-12, 1939, March 20, 1939, January 15-31, 1940, February 12-22, 1941; ♂, 2 ♀, Jupiter, February 1-24, 1920, March, 1931; ♂, 2 ♀, Biscayne Bay (A. T. Slosson); ♀, Lake Worth (A. T. Slosson); ♂, ♀, Indian River (collection of Henry Edwards).

The following fourteen paratypes, all from southern Florida, are in the collection of Mr. C. F. dos Passos: ♂, ♀, Miami, February 14, 1931; 2 ♀, Florida City, January 13, 1934; ♀, Key West, March 8, 1938; and from Key Largo, 4 ♂, December 20, ♂, December 27, 3 ♀, January 5, 8 and 13, ♀, February 26.

There are two paratypes in the collection of Mr. Harry K. Clench: ♂, East Titusville, Florida, March 17, 1939; ♂, Key Largo, Florida, March 30, 1939.

In the collection of the Museum of Comparative Zoology, Cambridge, Massachusetts, there are the following paratypes: ♂, Capron, Florida, April 1; ♂, Cocoanut Grove, Florida, June, 1929; ♂, Florida (no other

data); ♀, Paradise Key, Florida, April 1, 1926; 2 ♀, Indian River, Florida.

Grossbeck gave *Guilandia bonducella* and *Pithecolobium guadalupensis* as food plants of the larva.

This subspecies is named for G. T. Bethune-Baker, who recognized that this Floridian butterfly differed from *ammon*.

While we do not name the summer brood, it differs from the winter brood in that the marginal spots of the hindwing in the males are more prominent (but never so definite as in *ammon*) and the veins of both wings are slightly outlined with black scaling. The females of the summer brood have an even greater extension of the black-brown areas on the upperside than in *ammon*, with the blue coloring more limited to the basal areas. The underside does not differ greatly from the winter brood in either sex.

Hemiargus ammon noeli NEW SUBSPECIES

PLATE 1, FIGURE 23

Size and shape: the average size and wingshape does not differ from that of Cuban *ammon*.

Ground color: in the males, the blue of the upperside is like that of the summer brood of *ammon*, except that it is more dense; in the females, the upperside color is brownish black; on the underside of both sexes, the ground color is brownish gray as in *ammon*.

Markings: in males, on the upperside, the forewings have a brownish black outer border .5 mm. wide followed by a white fringe checkered with brown at the veins (in *ammon*, there is a narrow black border line with a similar fringe); the hindwings are as in *ammon*, except that the pink spot is absent and the black marginal spots are reduced in size, one or both sometimes absent; in females, on the upperside, the forewings have the basal half, including the cell area, metallic greenish blue (not bright blue as in the summer brood of *ammon*); the hindwings resemble those of *ammon*, but are darker, having much less blue color and the orange capping of the marginal black spot anterior to Cu_2 is no longer than that spot. The underside is similar in the sexes and the pattern is as in *ammon*, but modified in the following ways: in the forewing, the postmesial white band is reduced to small sagittate spots (quadrate in *ammon*); in the hindwing, the postmesial white band is made up of a somewhat uniform series of somewhat bottle-shaped spots placed in the interspaces and well separated from each other (in *ammon* these spots are subquadrate and separated slightly by the brown scaling on the veins); the black, white-ringed spots at the base are four

in number, that one near the base, between Cu_2 and 2A, being present (this latter one is usually absent in *ammon*); the marginal black spot anterior to Cu_2 is narrowly capped with orange for less than its diameter; it does not disarrange the postmesial band (in *ammon* this cap is red-orange, elliptically elongate basad, and the spot of the postmesial band anterior to Cu_2 is shifted basad).

In general appearance, *noëli* is a darker butterfly than *ammon*, and in specimens taken from January to August, no seasonal modification was noted.

Types, all from Hispaniola: holotype, male and allotype, female, St. Marc, Haiti, March 30–April 2, 1922. Paratypes: 3 ♂, 3 ♀, St. Marc, Haiti, March 30–April 2, 1922; ♂, 2 ♀, Aux Cayes, Haiti, March 15–20, 1922; ♂, Bizoton, Haiti, January 9, 1922; ♀, Carrefour, Haiti, April 8, 1922; ♂, Fond Parisien, Haiti, February 11–18, 1922; ♂, near Fort Liberty, Haiti, July 14, 1935; ♀, La Serre, Haiti, March 3, 1922; ♂, Pétionville, Haiti, May 23, 1930; 6 ♂, 4 ♀, Port-au-Prince, Haiti, various dates, January 14–April 8; ♀, Trouin, Haiti, April 4, 1935; 2 ♂, Barahona, Dominican Republic, July 6–11, August 10–15, 1932; ♀, Bonao to La Vega, Dominican Republic, March 16, 1931; ♂, Sanchez, Dominican Republic, May 11–16, 1915.

In the collection of the Museum of Comparative Zoology, Cambridge, Massachusetts, there are the following paratypes: 2 ♂, Camp Perrin, Haiti, October 9, 1934; 3 ♂, Haiti (P. R. Uhler); ♂, "San Domingo," A. G. Weeks' collection.

The following paratypes are in the collection of Cornell University, Ithaca, N. Y.: 2 ♂, ♀, Monte Cristi, Dominican Republic, June 7, 1916, collector C. P. Schmidt.

A single male taken by Dr. F. E. Lutz on Mona Island, W. I. (located in Mona Passage), February 21–26, 1914, is referable to this subspecies.

This subspecies is named for V. Pierre-Noël, Haitian artist, who has illustrated many entomological papers.

Hemiargus ammon woodruffi NEW SUBSPECIES

PLATE I, FIGURE 24

Size and shape: the length of the forewing, in both sexes, averages about 11.5 mm. for specimens from the Virgin Islands and 12.3 mm. for four males from Puerto Rico; the wing expanse is thus more than in *ammon* and about the same as in *bethune-bakeri*. The wing shape is as in all subspecies of *ammon*.

Ground color: the males, on the upperside, are a purplish blue, duller and denser than in other subspecies of *ammon*; the females are brown-

ish black; both sexes, on the underside, have a brown ground color which is less grayish than in any other subspecies and the more noticeable because of the reduction of the white markings.

Markings: the males, on the upperside, have a narrow marginal line of brown followed by an obscure white fringe and possess the two characteristic marginal spots on the hindwing, which are of about equal size and distinctness; the females have a slight basal suffusion of dull purplish blue on both wings; in the hindwings, distad of the basal blue area, there is a line of indefinite white spots and, submarginally, traces of arcuate white spots; the marginal black spot anterior to Cu_2 is capped with a red-orange spot as elongate and brilliant as in summer brood *ammon*, but rounded basad (not pointed basad as in *ammon*); on either side of this orange spot, there are traces of orange in the adjoining cells; the anal spot is capped with a white crescent more obvious than that in *ammon* because it contrasts with the brown ground color. The underside of both sexes differs from all other subspecies of *ammon* in that the white markings are much reduced; in the forewing, the post-mesial white band (so fully developed in *bethune-bakeri* and present in other subspecies) is almost wanting because of the shifting distad of the dark mesial line; in the hindwing, partly as a result of a similar shift distad, the white spots of the mesial row are narrowed and the postmesial white band is greatly narrowed, so that the brown ground color appears as wide stripes between the white markings; the two marginal black spots of the hindwing are of about equal size, the anal spot capped with a white crescent, and the black spot anterior to Cu_2 with a cap of red-orange as long as the diameter of the black spot; the subcostal black spot and the four basal black spots, ringed with white, are prominent.

Types: holotype, male and allotype, female, Aneгада, B. V. I., March 31, 1925. Paratypes: ♀, Aneгада, March 31, 1925; 4 ♂, Tortola, B. V. I., April 2, 1925; 2 ♂, St. Thomas, V. I., February 22, 1925; 2 ♂, St. John, V. I., March 6, 9, 1925; 4 ♂, Puerto Rico, "Aguareello," December, 1908, San Juan and "Santos," no dates.

The following paratypes are in the collection of Cornell University, Ithaca, N. Y.: 2 ♂, Basse Terre, St. Kitts, B. W. I., March 26, 1927.

This subspecies is named for the late Lewis B. Woodruff, who collected many of the specimens including holotype and allotype.

Hemiargus dominica (Möschler)

Lycaena dominica Möschler (1886) 26, fig. 10. Jamaica.

Hemiargus ammon f. *dominica*, Draudt (1921) 820.

Leptotes dominica, Kaye (1931) 584, pl. 39, figs. 1, 4, 7.

Draudt had probably never seen *dominica* or he would not have

called it an "insignificant form" or *ammon*; further, he gave the locality incorrectly, as Kaye pointed out. The species is known only from Jamaica, and Draudt's locality, Dominica, B. W. I., would seem to be an error. Dr. Avinoff informs us that he has not observed *ammon* in Jamaica, although he has captured *dominica* frequently.

We have seen both sexes from Baron Hill, Trelawny, and Claremont, Jamaica, taken in March, May and June, where it flew with *cassius theonus* and *perkinsae* Kaye. The species is distinct, its pattern being well shown for the male by Möschler and for both sexes by Kaye.

An examination of *dominica* shows that it is a member of the *ammon* group, as indicated by Draudt. In the males, the presence of the pink spot basad of the marginal black spot anterior to Cu_2 shows a similarity to Cuban *ammon*. In both sexes the underside pattern, although modified, is of the *ammon* type. We therefore place *dominica* as a distinct species in the genus *Hemiargus*.

Hemiargus bahamensis Clench

Hemiargus bahamensis Clench (1948) 57. Bahamas.

This species was described from a single male captured on Crooked Island, Bahamas.

Hemiargus isola isola (Reakirt)

Lycaena isola Reakirt (1866) 332, No. 28. Mexico.

The type was a female described from "Mexico (near Vera Cruz)."

A fresh male of *isola* was taken by Mr. F. E. Watson, about three miles east of the railroad station along the tracks at Tallaboa, near Ponce, Puerto Rico, July 23, 1914. The location was dry, with vegetation of *Cactus* and *Acacia*. It may be noted that this locality is on the southern, or Caribbean side of the island.

The specimen is typical *isola* and can be matched absolutely by males from the type region of eastern Mexico, such as Jalapa or Coatepec. This is of interest, as *isola* is locally variable and specimens from other Mexican and Central American regions show differences.

However, we cannot regard this specimen of *isola* from Puerto Rico as other than of casual occurrence, for it is the only record known to us.

Hemiargus bornoi NEW SPECIES

PLATE 1, FIGURES 18, 19

Size and shape: the length of forewing in six males varies from 10.9 mm. to 11.3 mm., with an average of 11 mm.; in six females from 11 mm. to 13 mm., with an average of 12 mm.; there is a tail at Cu_2 , the longest measured in males being 2.5 mm. and, in females, 3.5 mm.;

otherwise, the wings are shaped as in other species of *Hemiargus*, except that they have a very slightly produced anal lobe. In spite of the thecline character of the hindwing, vein R_4 is present in the forewing.

Ground color: in males, the upperside is gray-brown with a faint sheen of pale lavender, most noticeable along the costa of the forewing; in females, the gray-brown color is darker with a violet-blue coloring of greater brilliance in the forewing, which covers the cell and the space below, extending to the inner margin with a metallic greenish lustre at the base; the hindwing, in its posterior half, is whitish violet-blue, variable in intensity in the series of specimens; the underside of both sexes is grayish white.

Markings: on the upperside, in the forewing, there is a short black bar at the end of the cell, obsolete in males but distinct in females; in the hindwing, there is an oval black marginal spot anterior to Cu_2 as wide as the cell and a smaller marginal spot in each of several other cells; the fringes on both wings in both sexes are white. The underside, in both sexes, has the typical *Hemiargus* pattern, but modified in the following manner: in the forewing, there is a brown bar defined by white margins midway of the cell, in addition to the bar which closes the cell; the spots which form the mesial line are isolated and encircled with white (not merged and laterally bounded with white as in other *Hemiargus*) and as widely separated from the submarginal spots as in *bethune-bakeri*; the submarginal spots are small and clearly defined (not merged). In the hindwing, the pattern shows some slight rearrangement of maculation; the two black, white-ringed spots below the costa are closer together and there is a third similar spot behind the base of Sc (not present in other *Hemiargus*); beyond the broad post-mesial white band, and occupying the submarginal area completely from M_3 to the anal angle, there is a yellow field upon which the submarginal maculation is in part placed; this marking consists of a row of dark brown and black spots, provided with more or less metallic golden green scaling, placed between veins along the entire outer margin; in this row, there is a prominent, rounded, black spot anterior to Cu_2 which is encircled with the golden green color.

Types, all from Hispaniola: holotype, male and allotype, female, Pont Beudet, Haiti, 100 feet, March 3-4, 1922. Paratypes: 5 ♂, 3 ♀, Pont Beudet, Haiti, 100 feet, March 3-4, 1922; ♀, Pétionville, Haiti, May 23, 1930; ♀, Freres, Haiti, May 30, 1930.

This species is named for Louis Borno, a president of the Republic of Haiti.

One specimen from Ensenada, Puerto Rico, June 14-19, 1915, is considered to be of this species.

The *hanno* group

In studying the extensive series of specimens before us, to which the name *hanno* (Stoll) is applied in a broad sense, we are impressed by the great variety of major and minor variations which occur in both the insular and continental populations. The many localities from which we have specimens of *hanno* indicate a distribution from Peru and Brazil throughout northern South America, Central America, the Antilles and the southern portion of North America. The discussion by Bethune-Baker (1916) of *hanno* and its relatives is not satisfactory and the synonymy given by Draudt (1921) is not helpful. Holland (1931: 270) is quite mistaken in his determination of *filenus*, our discussion of which is under *Hemiargus ammon*. We shall confine the present discussion to the Antillean populations, bringing in the continental populations only as necessary to show relationship, but with the intention of discussing these continental populations in a subsequent paper. The existing names to be considered are as follows:

- 1790. *Papilio hanno* Stoll, ♂, Surinam, Cape of Good Hope.
- 1793. *Hesperia ceraunus* Fabricius, ♀, "Americae meridionalis Insulis."
- 1812. *Rusticus adolescens hanno* Hubner, ♂, ♀, no locality.
- 1818. *Hemiargus antitubastus* Hübner, ♂, Georgia.
- 1832. *Polyommatus filenus* Poey ♂, ♀, Cuba.
- 1833. *Argus pseudoptiletes* Boisduval and LeConte, ♂, ♀, Georgia.

We believe that it is essential to consider the origin of a described species to properly recognize it, especially when dealing with a species having populations which vary subspecifically. Much confusion has heretofore occurred because of a disregard of type localities on the part of various authors, which has resulted in misidentifications and improper synonymy.

† *Hemiargus hanno hanno* (Stoll)

The information given by Stoll about this species is inconclusive, but subsequent authors have concurred in considering it an American species. Therefore, accepting Surinam as the type locality, we have examined specimens from that region and selected a male from Paramaribo, Surinam, as typical of the species. The figures given by Stoll are very crude and the determination of the species must be arbitrary but, as there seems to be no other species from the type region which better

conforms with the figures, our selection seems reasonable to us. Our determination does not differ from the accepted understanding of the species, except that we restrict the name *hanno hanno* to the Surinam population and those inseparably similar populations which spread through the Guianas and into Brazil.

We place *hanno* (Hübner) as a synonym as well as a homonym of *hanno* (Stoll). Hübner's beautiful figures exactly represent the typical *hanno* of Surinam, and Hübner's further action in the Verzeichniss should be conclusive, for he there indicated that he considered his species to be the same as Stoll's. We disagree with the actions of Kirby (1908, 3: 72) and Bethune-Baker (1916: 455) in making *Rusticus adolescens hanno* Hübner a synonym of *ceraunus* (Fabricius) which is, according to the Fabrician description, a different butterfly.

We do not know of satisfactory figures of typical *hanno* other than the figures of Hübner in the original Sammlung not available to many readers, and we therefore characterize it to distinguish it from what we consider subspecifically related forms.

The length of the forewing is variable in both sexes, being from 9 mm. to 10.5 mm., but the average is slightly less than 10 mm. In males, the upper surface is bright violet-blue with a black-brown outer margin, a little more or less than .5 mm. wide, variable in individuals, followed by a white fringe. In the Cu_1 to Cu_2 interspace, at the margin of the hindwing, there is no black spot in the majority of specimens; however, a few specimens show traces of such a spot. In females, this spot is faintly present. The females have a dark brown ground color with a faintly bright blue basal suffusion, and fringes which are brownish, with a few white scales in the forewing, and all white in the hindwing.

On the underside, *hanno hanno* has the characteristic *Hemiargus* pattern. The markings to be noted particularly for subspecific differentiation occur in the hindwing: the mesial row consists of oblong brown spots which are inwardly and outwardly bounded with white; distad of this row, there is a row of white sagittate spots, pointing basad and often obscure because of the surrounding whitish scaling. The sagittate spots are short, no longer than wide, and their points either do not touch or just touch the outwardly bounding white lines of the mesial spots. At the margin, in the Cu_1 to Cu_2 interspace there is a round black spot about .5 mm. in diameter, containing a few metallic scales distad and a narrow yellowish iris the width of which is less than half the diameter of the black spot. In the Cu_2 to 2A interspace there are two minute, somewhat oblong, brown spots surrounded with white,

which usually do not show any black or metallic scaling, but, if this is present, it appears in that spot nearest the anal angle. The females are similar to the males but usually a little more brownish in ground color.

Known to us from many islands of the lesser Antilles and also from Puerto Rico, there is a series of populations, closely related to each other, but easily distinguishable as a group from *hanno hanno*. We have chosen to select specimens from Puerto Rico as the types of this widespread subspecies.

Hemiargus hanno watsoni NEW SUBSPECIES

PLATE 1, FIGURE 20

Size and shape: the average length of the forewing for 20 males is 10.5 mm., ranging from 8.7 mm. to 12 mm.; for 20 females the average is 9.5 mm., ranging from 8.5 mm. to 11.5 mm. The size and wingshape is not different from *hanno*.

Ground color: on the upperside, the males are violet-blue and the females dark brown, neither sex to be separated from *hanno*; on the underside, both sexes are slightly darker than *hanno*, a result of the reduction in the dusting of white scales.

Markings: the males have a black-brown outer margin, individually variable in width as in *hanno*, with white fringes; as in *hanno*, there is no black spot present in the Cu_1 to Cu_2 interspace in the majority of the specimens, but when this does occur, it appears as a slight thickening of the border. In females, there is a slight basal suffusion of blue at the wing bases, as in *hanno*, generally most evident in the forewings; the black spot in the Cu_1 to Cu_2 interspace is obscurely present in many specimens; the fringes of both fore- and hindwings are white, thus differing from *hanno*. On the underside, the sexes do not differ in pattern, which is similar to that of *hanno* except in the following ways: in the hindwing in the postmesial band, the sagittate white spots are consistently joined to the white bars which distally bound the mesial brown spots; these postmesial white spots are not longer than those in *hanno* but are better defined because the brown band distad of them is not suffused with white scales as in *hanno*; the round black spot in the Cu_1 to Cu_2 interspace is about 1 mm. wide (twice the size of that in *hanno*), and the yellow iris is almost reduced to a lunate cap basad of the black spot; in the Cu_2 to 2A interspace, there are two brown spots, as in *hanno*, which, in less than 10 per cent of the 117 specimens examined from Puerto Rico, contain traces of black or metallic scaling.

Specimens caught throughout the year showed no constant seasonal difference.

Types, all from Puerto Rico; holotype, male and allotype, female, San Juan, July 9-12, 1914. Paratypes: San Juan, 3 ♂, 3 ♀, February 9, 11-14, 1914, 6 ♂, 6 ♀, July 9-12, 1914, ♂, August 19, 1907; ♂, Adjuntas, June 13, 1915; Aibonito, ♂, July 14-17, 1914, ♀, June 1-3, 1915; 3 ♂, ♀, Arecibo, July 30-August 1, 1914; ♂, Bayamon, December 19, 1907; ♀, Cataño, January 3, 1914; 2 ♂, ♀, Coamo Springs, July 17-19, 1914; ♂, Ensenada, June 14-19, 1915; 3 ♂, ♀, Guayanilla, July 22, 1914; ♂, Guánica, August 18, 1907; ♀, Mameyes, November 19, 1925; 3 ♀, Mayagüez, July 24-29, 1914, November 14, 1925; ♀, Naguabo, March 7-9, 1914; ♂, Tallaboa, March 7, 1927.

This subspecies is named for Mr. Frank E. Watson, who collected many of the specimens and recognized that the population of *hanno* in Puerto Rico represented a different form.

We have seen specimens from the Virgin Islands, St. Kitts, Antigua, Guadeloupe, Dominica, Martinique, St. Lucia, Grenada and Barbados. We have observed minor variations among them; but we feel, for the present, that the definition of the subspecies *watsoni* from Puerto Rico is sufficient to include them.

The subspecies of *hanno* which occur in the islands of the Greater Antilles other than Puerto Rico are definitely separated as a group, by a difference in pattern, from the populations of Puerto Rico and the Lesser Antilles. These populations we regard as two subspecies, the first of which occurs in Hispaniola and Jamaica.

Hemiargus hanno ceraunus (Fabricius)

Hesperia ceraunus Fabricius (1788) 303, No. 149. "Americae meridionalis Insulis."

Lampides ceraunus, Butler (1869) 163; (1878) 481. Jamaica.

Hemiargus ceraunus, Kirby (1908) 1, pl. 98, figs. 1-4; 3, 72.

Hemiargus ceraunus, Bethune-Baker (1916) 455.

Fabricius gave sufficient information about *ceraunus* in his description to make it clear that the butterfly he had before him was a female and that it was some subspecies of *hanno* from the Antilles. He further said that it had black marginal spots on the upperside of the hindwing which further limited its origin to either Cuba, Hispaniola or Jamaica.

Butler, in his study of the Fabrician species and in his later paper on Lepidoptera from Jamaica, considered that *ceraunus* occurred in Jamaica. We agree with Butler's decision but, in addition, consider the population of Hispaniola to be *ceraunus*.

Kirby accepted *ceraunus* but incorrectly placed *hanno* (Hübner) as a synonym. Kirby's figures are so very poor that they cannot be said to

represent any species in particular. Bethune-Baker followed Kirby into the same error of synonymy.

The subspecies *ceraunus* may be separated on the upperside from *hanno* and *watsoni* by the increase, in both sexes, in the size of the black spot in the Cu_1 to Cu_2 interspace. This spot is not consistently large and prominent, but it is at once noticeable in the large series of specimens we have examined. On the underside, the similarly placed spot is elongate basad (not round as in *hanno* and *watsoni*), and therefore larger; in the postmesial row, the white sagittate spots are lengthened, and in many specimens the spots have lost their sagittate shape; the two spots in the Cu_2 to 2A interspace are more frequently filled in with black and metallic scales. A count made on 122 specimens of *ceraunus*, from Hispaniola, showed that 37 specimens or 30 per cent of them have some trace of black and metallic scaling. A similar count on Jamaican specimens showed only 10 out of 46 or 22 per cent with this scaling. These pattern changes were apparently not seasonal or local differences, for the series came from different localities and the captures were made at all seasons of the year. There is no significant change in size in the case of *ceraunus*, for in the Jamaica and Hispaniolan series the average wing length corresponds with *watsoni*.

Hemiargus hanno filenus (Poey)

Polyommatus filenus Poey (1832) No. 18, 3 figs. Cuba.

Polyommatus philenus Poey (1852) 197.

Hemiargus filenus, Bates (1935) 196.

This subspecies of *hanno* occurring in Cuba is magnificently figured by Poey and well described. Its determination is in no way difficult, if a copy of Poey is available, yet it has been grossly misdetermined by a number of authors. Poey's description is interesting, for under the heading "Observations," he describes an unnamed Cuban butterfly, later to be described by Lucas (1857) as *ammon*. Poey's second reference reads: "It seems better to me to write *philenus*."

We place *filenus* as a subspecies of *hanno*. It is the most differentiated from typical *hanno* of the Antillean populations. In it, the black spot on the upperside of the hindwing is larger than in any other subspecies; on the underside the postmesial sagittate spots are more elongate, joining completely with the outer white mesial bars, and they are wider, forming a white postmesial band interrupted by brown lines along the veins; the black spot in the Cu_1 and Cu_2 interspace is an elongate oval, definitely larger than in *ceraunus* and twice the size of *watsoni*; the two spots in the Cu_2 to 2A interspace show black and metallic scales in 37 out of 54 specimens or in 70 per cent of the specimens.

Dethier (1940: 24) described the egg and first instar of *flenus* and gave *Mimosa pudica* as the food plant. Haskin (1933: 155) (as *hanno*) gave further life history notes.

The Antillean subspecies of *hanno*, in their progression through the Lesser and Greater Antilles, present a definite subspecific cline. We recognize, in a number of given characters, a succession of changes from one population to another, such as, for example, the gradual increase in the length of the sagittate spots. These changes occur in minute, progressive steps from island to island in the Lesser Antilles and in more pronounced steps west of Puerto Rico in the Greater Antilles.

Hemiargus hanno antibubastus Hubner

Hemiargus antibubastus Hubner (1818) Zutrage, 1, 19, figs. 99, 100. "Aus Georgien in Florida."
Argus pseudopteleus Boisduval and LeConte (1838) 114, pl. 35, figs. 5-7. "Il se trouve dans la Caroline, la Virginie, la Géorgie, dans plusieurs Antilles et au Mexique."
Hemiargus antibubastus, Bethune-Baker (1916) 452.
Lycaena hanno, Holland (not Stoll) (1931) 270, pl. 32, fig. 8.

This subspecies, of common occurrence in southern Florida and in the southern states, is figured by Holland, showing the underside, not overclearly, but sufficiently to make its pattern recognizable. A comparison of the male genital armature with that of *flenus* shows that the two subspecies cannot be separated by this structure. We believe that *antibubastus* is a subspecies of *hanno* which has been separated from the Antillean populations, as a whole, for a very long time, for it is distinctly different from all of the others and its pattern is modified in different ways from those in the island cline. The size of *antibubastus* is another definite change. Although occasional specimens occur which are no larger than *flenus* and other insular subspecies, the average size is much greater. Males and females with a forewing length of 13 mm. are not uncommon and some males reach 13.5 mm., females 14.5 mm. The black spots on the upperside of the hindwings are usually prominent in both sexes; the females tend to have a more extensive and brighter blue suffusion, in some specimens covering four-fifths of the wing areas. The underside has a pronounced gray-brown ground color upon which the gray-white pattern is clear; the sagittate spots of the postmesial row are suffused but usually detached from the outer white bars of the mesial row; the black spot in the Cu₁ to Cu₂ interspace is basally elongate and prominent and the spots of the Cu₂ to 2A interspace are occasionally present. A divergence from the typical *hanno* pattern occurs in the row of submarginal lunate spots on the underside of the hindwing. These lunules are very obscure, and finely traced on the brown ground color. The whole appearance of the butterfly is distinctly different from that of the insular subspecies of *hanno*.

We have recognized this species as *antibubastus* from Hübner's de-

scription and figures, with the aid of certain specimens selected from a series of 150 specimens from Georgia, Florida and Alabama. The males are quite variable, in that the outer brown border may be broad with a pronounced marginal black spot in the hindwing, or the border may be a narrow line with the black spot absent. Hübner's figure No. 99 is of the latter type. On the underside, the pattern is variable in intensity, and in some specimens the mesial row of spots are very dark, as shown in Hübner's figure No. 100. In this figure, the black spot with a yellow lunule in the Cu_1 to Cu_2 interspace is not shown, but Hübner mentions it in his text on page 20.

The butterfly figured by Boisduval and LeConte as *pseudoptiletes*, the name of which they corrected to *filenus* in their text, we consider to be a synonym of *antibubastus*.

The food plant, *Macroptilium lathyroides*, was given for *antibubastus* (as *hanno*) by Grossbeck (1917) and Wolcott (1941) gave the same foodplant for *watsoni* (as *filenus*), with the further note that the larva feeds upon the buds, flowers and seeds.

BREPHIDIUM Scudder

Brephidium Scudder (1876) 123.

Genotype.—*Lycæna exilis* Boisduval, 1852.

* *Brephidium exilis exilis* (Boisduval)

Lycæna exilis Boisduval (1852) 294. California.

Lycæna fea Edwards (1871) 211. Waco, Texas.

Brephidium exilis, Comstock (1927) 178, pl. 53, figs. 5-9.

Boisduval described this species from a single female without particular locality other than California. Edwards described *fea* from several specimens of both sexes taken near Waco, Texas, and said that it was allied to *exilis* but did not say how it differed. Comstock gave the range of *exilis* in California from the Sacramento Valley southward, Hoffman (1940: 721) gave Mexican records and Godman and Salvin, in the *Biologia* (1887, 2: 109), gave records from Guatemala and Venezuela. The occurrence of *exilis* is thus indicated from northern South America, through Central America, and well to the north in California. What we consider to be typical *exilis* is well figured by Comstock.

The life history of *Brephidium exilis*, with a list of food plants, was given by Coolidge (1924: 115).

Brephidium exilis isophthalma (Herrich-Schäffer)

Lycæna isophthalma Herrich-Schäffer (1862) 16, 141; (1864) 13, 164. Cuba.

Brephidium isophthalma, Bates (1885) 188.

A portion of the original description, translated, reads: "Upperside

with very reduced dark violet and white undappled fringes." Both *exilis* and *isophthalma* have a character in common in the fringe of the forewing which was apparently overlooked by Herrich-Schäffer. On the upperside, just anterior to Cu_2 , the fringe is brown, but even before Cu_1 is reached, in many specimens, there is an intermingling of white scales. Otherwise, the fringe is made up of brown scales near the margin and slightly longer white ones outwardly. Fresh specimens are necessary to note this characteristic. The subspecies *isophthalma* may be distinguished from *exilis* by the pattern of the underside of the forewing, where the two submarginal rows of elongate white spots are distinct and are as well defined as the spots of the mesial line. (In *exilis* these submarginal spots are usually faint, the inner row frequently obsolete, and the spots of both rows less clearly defined than the spots of the mesial line.)

The male genitalia of specimens from California and Hispaniola appear alike, with an unusual development of organs which correspond with the characteristic falces of the Lycaenidae. The tegumen is enlarged on either side and, from this structure, there extend two truncate processes which carry, terminally, six or seven strong spines.

We recognize specimens from Cuba and Hispaniola as belonging to the subspecies *isophthalma*. We have never seen this subspecies from Florida. Clench (1943: 58) recorded *isophthalma* from New Providence, Bahamas.

Dr. A. Avinoff was the first to note the occurrence of the species *exilis* in Jamaica. For the present, we refer these specimens to the subspecies *isophthalma*.

Brephidium barbouri Clench

Brephidium barbouri Clench (1943) 58. Bahamas.

This species was described from specimens taken on Great Inagua and Rum Cay in the Bahamas. The author stated verbally that it is intermediate between *isophthalma* and *pseudofea* in color and pattern.

Brephidium pseudofea (Morrison)

Lycaena pseudofea Morrison (1873) 186. Florida.
Lycaena isophthalma, Holland (not Herrich-Schäffer) (1898) 269, pl. 32, fig. 4, ♂.

Morrison described *pseudofea* from three specimens taken at Key West, Florida. He sufficiently characterized his species and compared it with *exilis* and *fea*, pointing out the differences. He was probably unaware of Herrich-Schäffer's description of *isophthalma*, or he could easily have made a further distinction from that Cuban butterfly and prevented years of misapplication of that name.

Morrison says of *pseudofea*: "Wings above uniform dark brown, deeper at the base, and there concolorous with the body; anterior wings without defined markings; posterior wings with a series of five small submarginal black spots, the three nearest the anal angle distinct, the other two sometimes almost obliterated; fringe concolorous, without any trace of white." The underside differences are also clearly pointed out.

Further characters which separate *pseudofea* from the subspecies of *exilis* and cause us to consider it a distinct species, are the more elongate forewings and a distinction in the genitalia. The tegumen, where laterally enlarged, is more robust and further expanded than the similar structures in *exilis* and *isophthalma*. Otherwise, the genital armatures of the males in the two species are of generally similar structure.

We know *pseudofea* from Florida and we have one specimen labeled "Galveston, Tex. May. F. H. Snow," determined as *isophthalma* by Dr. Henry Skinner. Under the name *isophthalma*, as it has been determined by many authors, it is recorded from Georgia and the Gulf States. Harry K. Clench has independently recognized the specific position of *pseudofea* and will comment thereon in a forthcoming article.

Since the submission of our manuscript, an article on the Lepidoptera of the Cayman Islands by Carpenter and Lewis has appeared. In this, the following species of Lycaenidae are listed:

Strymon martialis (Herrich-Schäffer).

Strymon acis (Drury). This is probably not *acis acis* but a subspecies.

Strymon columella (Fabricius). We would be inclined to associate this with *T. columella cybira* Hewitson.

Hemiargus filenus (Poey). This may not be typical *H. hanno filenus* which we recognize from Cuba. It may well be *H. hanno ceraunus* (Fabricius) occurring in Jamaica and Hispaniola, or a distinct subspecies.

Hemiargus ammon (Lucas). This may not be the typical *ammon ammon* of Cuba.

Hemiargus catilina (Fabricius). This determination does not agree with our conception of *catilina* which we place in the genus *Leptotes*. From the information given, we are inclined to believe that the Cayman specimens belong in the *ammon* group and quite possibly may represent a distinct subspecies.

Briphidium exilis thompsoni Carpenter and Lewis. This is an interesting discovery in the *exilis* group. We have commented at length on *exilis* and its allies in the preceding pages.

Leptotes theonus (Lucas). Considering the general Antillean distribution of *cassius theonus*, this may be that form.

DISTRIBUTION

In explanation of the accompanying distribution charts (TABLES 1 AND 2), the localities listed are those from which we have seen speci-

TABLE I
DISTRIBUTION OF THE THECLINAE

THECLINAE	North Am. East	Southern Florida	Dry Tortugas	Bahamas	Cuba	Jamaica	Hispamiola	Puerto Rico	Virgin Island	St. Kitts	Antigua	Guadeloupe	Dominica	Martinique	St. Lucia	St. Vincent	Grenada	Barbados	Trinidad	South America	Central America	North Am. West
<i>Eumecurus atala grapti</i> G. & H.	E	X			X																	
<i>atala atala</i> (Poey)	E																					
<i>Thecla marsyas cybele</i> G. & S.	E																			X	X	
<i>Thecla marsyas marsyas</i> (Linnaeus)	*																		X	X		
<i>Thecla cecrops</i> (Fabricius)		X																				
<i>Thecla beon</i> (Cramer)							?															
<i>Thecla bourkei</i> Kaye	E					X																
<i>Thecla piplea</i> G. & S.	E												X			X						
<i>Thecla pan</i> (Drury)	E					X																
<i>Thecla rufo-fusca</i> Hewitson																X					X	
<i>Thecla roelofs</i> H.-S.	E				X		X	X														
<i>Thecla fulena</i> Hewitson	E																					
<i>Thecla angerona</i> G. & S.	E									X			XX									
<i>Thecla dominicana</i> Lathy	E																					
<i>Thecla celida celida</i> Lucas	E																					
<i>Thecla celida shawmatalfi</i> G. & H.	E				X																	
<i>Thecla celida albomilo</i> G. & H.	E					X																

TABLE 1 (Continued)

	North Am. East	Southern Florida	Dry Tortugas	Bahamas	Cuba	Jamaica	Hispaniola	Puerto Rico	Virgin Islands	St. Kitts	Antigua	Guadeloupe	Dominica	Martinique	St. Lucia	St. Vincent	Grenada	Barbados	Trinidad	South America	Central America	North Am. West
<i>Thecla angelia dowi</i> (Clench)				X	X																X	X
<i>Thecla angelia angelia</i> Hewitson							X	X													X	X
<i>Thecla angelia pattoni</i> C. & H.																					X	X
<i>Thecla angelia boycei</i> C. & H.																					X	X
<i>Thecla favonius</i> (A. & S.)																						
<i>Thecla marialis</i> H.-S.	X	X		X																		
<i>Thecla acis bartolami</i> C. & H.		X			X	X																
<i>Thecla acis armouri</i> (Clench)				X																		
<i>Thecla acis rasasi</i> C. & H.					X																	
<i>Thecla acis gossii</i> C. & H.					X	X																
<i>Thecla acis peironi</i> C. & H.																						
<i>Thecla acis mars</i> (Fabricius)										X			?									
<i>Thecla acis aris</i> (Drury)																						
<i>Thecla maesiles maesiles</i> H.-S.		X		X	X	X		X	X												X	X
<i>Thecla maesiles clenchi</i> C. & H.													X								X	X
<i>Thecla maesiles telca</i> Hewitson																					X	X
<i>Thecla sinuelthis sinuelthis</i> (Drury)					X	X																
<i>Thecla sinuelthis jugo</i> C. & H.																						
<i>Thecla crithona</i> Hewitson																						

TABLE 2
DISTRIBUTION OF THE PLEBEJINAE

PLEBEJINAE	North Am. East	Southern Florida	Dry Tortugas	Bahamas	Cuba	Jamaica	Hispaniola	Puerto Rico	Virgin Islands	St. Kitts	Antigua	Guadeloupe	Dominica	Martinique	St. Lucia	St. Vincent	Grenada	Barbados	Trinidad	South America	Central America	North Am. West
<i>Leptotes cassius theomus</i> (Lucas)	..	X	..	X	X	X	X	X	X	X	..	X	X	X	X
<i>Leptotes cassius catilina</i> (Fabricius)	X	X	X
<i>Leptotes cassius chadwicki</i> C. & H.
<i>Leptotes cassius cassius</i> (Cramer)
<i>Leptotes cassius striata</i> (Edwards)
<i>Leptotes perkinsae</i> Kaye
<i>Leptotes marina</i> (Reakirt)	X	..	?
<i>Hemiarqus amnon bellune-bakeri</i> C. & H.	..	X
<i>Hemiarqus amnon thomasi</i> Clench	X	X	X
<i>Hemiarqus amnon amnon</i> (Lucas)
<i>Hemiarqus amnon noëti</i> C. & H.
<i>Hemiarqus amnon woodruffi</i> C. & H.
<i>Hemiarqus dominica</i> (Möschler)

mens. These localities are arranged so that the progression is from eastern North America southeasterly through the Antilles to South America. Central America and western North America, the continental parallel to the insular chain, are in the last two columns. The heavier vertical lines separate the continents, the Greater Antilles and the Lesser Antilles. The species and subspecies are arranged so that the distribution of the forms from west to east may be easily noted. Those which appear to be closely related are grouped together. This arrangement is not intended to be that of a check list, nor does it follow the arrangement of the systematic account. In the first vertical column of a chart, the letter "E" indicates that, from our present knowledge, a species or subspecies is endemic in a particular region or group of regions indicated in the body of the chart. In the same column, an "*" indicates that the species or subspecies is extralimital. In the body of a chart, an "X" indicates that a form is known from a particular locality; an "o" indicates that we consider that a form may be of only casual occurrence in a locality; a "?" indicates that we are in doubt of the determination or that we question the accuracy of the record for the locality. The number of forms in a given region is placed at the bottom of a column, separately for the Theclinae and Plebejinae, and in a combined total; the number of forms considered to be endemic in each region is placed below the totals.

The charts show that the known distribution of both the species and subspecies of the Lycaenidae in the entire Antillean region is broken and irregular. We suspect that much of this apparent irregularity is due to the limited data available for the preparation of the charts. More extensive collecting will undoubtedly provide records to fill the obvious gaps. Certain facts about the distribution of forms, however, are evident, even from the admittedly incomplete data, and it seems possible to summarize and to formulate some general conclusions as to the trends, relationships and composition of the populations of the species discussed.

The 76 forms discussed in the systematic account are under consideration and listed in the charts. Of these, there are 10 definitely extralimital forms that are included because of their relationship to Antillean forms; 2 of which the records are considered doubtful; and 2, which, for the present, are considered casuals. Excluding these 14 forms, there remain 62 distinct forms occurring in the Antilles and southern Florida, which we recognize as 36 species with an additional 26 subspecies, all of which are distinctly Neotropical. There is not one species which even suggests that it might be of Nearctic origin.

CONTINENTAL RELATIONSHIPS

The meagerness of this Antillean lycaenid fauna is shown by comparison with the faunas of surrounding areas. Florida, according to Grossbeck (1917), has 20 species of Lycaenidae. Hoffman (1941) lists 210 species from Mexico, and Godman and Salvin (1901), in the *Biologia*, list 235 species from Central America, including Mexico. From Trinidad, which has a small representation of the northern South American fauna, Kaye (1921) lists 58 species of Lycaenidae. The total of different species given in these lists is about 340, which is more than five times the combined number of species and subspecies occurring in the entire Antillean region. The poverty of the Antillean fauna is even more apparent, if the total of 36 species (excluding subspecies) alone is compared, in which case the continental fauna is more than nine times greater.

The extent of the relationships between the Antillean and continental faunas may be judged by an examination and comparison of the populations of the various nearby regions.

There are 4 species with their subspecies which are extensively distributed through the Antilles and all of the surrounding continental regions. These are *Thecla maesites*, *Thecla simaethis*, *Leptotes cussius* and *Hemiargus hanno*.

In contrast to the 4 species just mentioned, there are 3 species which occur in southern Florida, the Gulf States, and the southern Atlantic States (*Thecla cecrops*, *Thecla favonius* and *Brephidium pseudofea*), which are not recorded from other localities, but each of which has a close Neotropical relative and no close northern relative. Another species found in southern Florida (*Thecla martialis*) occurs without appreciable variation in several islands of the Greater Antilles. Four more species found in southern Florida are represented by various other subspecies in the islands of the Greater Antilles and elsewhere.

The close connection of 11 Lycaenidae, occurring in southern Florida, with the Greater Antillean fauna is very obvious, and it is our view that at least those 8 not found north of southern Florida arrived there from the Antilles and not the reverse. The time of their arrival is a matter of speculation, but it may be noted that the 4 forms which range beyond southern Florida are the most different in facies from the insular forms, perhaps indicating an earlier date for their arrival.

There are 11 forms noted on the charts from Central America, 3 of which (*Thecla beon*, *Leptotes marini* and *Hemiargus isola*) can be eliminated from this discussion because of the uncertainty of the records of the first two and the casual occurrence of the last. Of the re-

maining 8, 4 are the generally distributed species first mentioned. Of these, *Thecla maesites* is represented in both Central and South America by the subspecies *telea*; *Thecla simaethis* occurs extensively in the islands and in Central and South America, without appreciable change; *Leptotes cassius cassius* occurs in northern South America, *cassius striata* in Central America, and, in the islands, there are 3 additional subspecies; *Hemiargus hanno hanno* is typically South American, but occurs in Panama and appears throughout the islands and in eastern North America as a series of subspecies. Of the 4 species still to be mentioned, little is known of *Thecla rufo-fusca* beyond its occurrence in St. Vincent and in Central America; *Thecla columella*, with 4 subspecies distributed in the Greater Antilles and Florida, is represented in Central America by the subspecies *istapa*; and *Thecla bazochii* and *Brephidium exilis*, occurring in both Central and South America, are represented in the Greater Antilles by subspecies.

The Antillean-Central American relationships may be considered from two viewpoints: first, parallelism in development of forms of common origin occurring in similar latitudes; and, second, close genetic relationship of Antillean and Central American forms through migration across the Caribbean. Based on the facts presented in the systematic account and the distribution shown by the charts, we incline to the theory that a number of the Antillean Lycaenidae may be of Central American ancestry.

There are only 2 species, to our knowledge, which are limited to South America and the Lesser Antilles. One of these, *Thecla marsyas*, we know from Trinidad and northern South America; Godman and Salvin report one specimen from Panama, but the common form at the Isthmus and northward is *Thecla damo* which, for the present, we consider a separate, though closely allied species. In St. Vincent, there is the distinct and beautiful subspecies, *Thecla marsyas cybele*. The second species, *Thecla bubastus*, occurs, to our knowledge, only in northern South America and Trinidad, but the subspecies *bubastus ponce* extends to Puerto Rico, as indicated in the chart. The inference is that these 2 species entered the Lesser Antilles from South America. We have previously mentioned *Hemiargus hanno* because of its wide distribution, but it should be included here, for it seems reasonably evident that it also invaded the Antilles from northern South America. Another species, *Leptotes cassius*, we also consider to be of South American origin but, because of the nature of the evidence presented in the systematic account, we believe that it arrived in the Antilles from a Central American source.

INSULAR INTERRELATIONSHIPS

The fauna within the Antilles may be divided into two main groups: that of the Greater Antilles, containing 28 species, and that of the Lesser Antilles, containing 13 species, with but 7 species common to both. The dividing line taken between Puerto Rico and the Virgin Islands shows that there is a definite break with only a slight overlap in this region. Of the 13 species recognized as regular inhabitants of Puerto Rico, 12 are known in some form from the islands to the west. Of these, 6 do not range east of Puerto Rico, 2 stop in St. Kitts, and 4 are the generally distributed species. Only 1 form (*Thecla bubastus ponce*) ranges from Puerto Rico southeasterly with a distinctly Lesser Antillean distribution.

The fauna is much diversified, for, of the 62 forms known from southern Florida and the Antilles, there are only 13 that are known from three or more localities, and 4 of these forms belong to the generally distributed species.

The occurrence of series of subspecies in adjacent islands is frequent, as shown by the charts. Of the 36 species discussed, 14 divide into subspecies, some prolifically so. An interesting development of this marked production of subspecies in adjacent islands is the clinal tendency evidenced in the subspecies of *angelia*, *acis*, *columella*, *cassius* and *hanno*. There is a well-developed cline in *hanno* for, diverging from the typical form of the Guianas, there is a minutely graduated progression of changes in the various island populations of the subspecies *watsoni* occurring from Grenada to Puerto Rico. These changes are most easily noted in several elements of the pattern of the underside of the hindwings, and the population of Puerto Rico is the most divergent from that of the Guianas, both in the extent of the changes and the predominance in numbers of individuals in the population so modified. While the steps of the pattern-changes between the populations are very slightly evident in the Lesser Antilles, similar pattern-changes are more marked between *watsoni*, *ceraunus* and *filenus* occurring in the Greater Antilles.

A striking characteristic of this Antillean fauna is the predominance of endemic species in the region as a whole and in its separate elements. These endemic species are enumerated in two ways on the charts. In the first column, of the 62 forms (species and subspecies), 56 (90 per cent) are noted as being endemic in southern Florida and the Greater and Lesser Antilles. Of the 36 separate species 23 (64 per cent) are considered to be endemic in the same region. In the last transverse

line of the last chart, the figures below the totals for each separate region indicate the number of forms which occur only in that particular region. In total, there are 33 forms which are known from only a single region. These figures are surprisingly large and suggest to us that much of the fauna was of fortuitous arrival in the past; further, that most of the fauna has been isolated for a long time and that it contains many relict forms.

The following compilation (TABLE 3), taken from our charts, shows the number of forms common to any two localities in the Greater Antillean region. It is obvious that a few new records would materially affect these proportionate relationships, and such additions are to be expected. However, using the data we have, the results are interesting. The total number of forms and the number of endemic forms in each of the six localities is given

TABLE 3

FORMS COMMON TO ANY TWO LOCALITIES, TOTAL NUMBER OF FORMS AND NUMBER OF ENDEMIC FORMS IN EACH LOCALITY

	Puerto Rico	Hispaniola	Jamaica	Cuba	Bahamas	Southern Florida	Total No.
Puerto Rico	2*						13†
Hispaniola		5*					14†
Jamaica	3	5	9*				17
Cuba	4	5	7	6*			15
Bahamas	2	2	5	6	5*		11
Southern Florida	2	1	3	3	3	3*	11

* Indicates number of endemic forms.

† Includes only forms known to be of regular occurrence.

If the figures in combination for each locality are added together, six comparable sums are obtained: Puerto Rico—17; Hispaniola—19; Jamaica—23; Cuba—25; Bahamas—18; Southern Florida—12. Cuba shows the largest figure, which might be expected, as it is at the center of the region, subject to interchanges of populations with four adjoining areas. It is quite obviously not a center of dispersal.

Another interesting condition is indicated by the figures for Jamaica, Cuba and the Bahamas. Jamaica has 7 forms in common with Cuba, 5 with the Bahamas; Cuba has 6 forms in common with the Bahamas. This indicates a north and south distribution. Comparing another condition, Hispaniola has 5 forms in common each with Cuba and Jamaica and 2 forms in common with the Bahamas. This is an east and west distribution and evidently less forceful. We think that there

is a possible explanation of the preponderant north and south distribution, between Jamaica, Cuba and the Bahamas, through hurricane distribution (Darlington, 1938).

The prevalent course of West Indian hurricanes is from the southeast, thus striking Hispaniola before reaching Jamaica, Cuba or the Bahamas. However, the progress of the storm centers is slow, twelve miles an hour or less. Objects are picked up and distributed by the cyclonic wind, which is often over 100 miles an hour. In large hurricanes, high velocity winds may occur 200 miles or more from the center. We think that the time element is an important factor in the distribution of living animals. It might take five or six hours to carry an insect from Hispaniola to Cuba or one of the southern islands of the Bahamas, and twice as long to reach Jamaica. This is because the storm center moves slowly and an object would have to be carried by the hurricane for a relatively long time before it could be deposited. On the other hand, a storm center over Jamaica, or between Jamaica and Cuba, or on a route farther north, so that it would reach the Bahamas, might carry a specimen across from one island to another in an hour or less, because it would be carried directly by the rapid cyclonic wind. Whatever the conditions or their possibilities may be, it is a fact that Jamaica, Cuba and the Bahamas have more in common than do the other islands. Hurricane transportation at least suggests a cause.

Of the various island faunas, that of Jamaica presents some of the most interesting Lycaenidae. There are 17 species known from Jamaica, the largest number from any island, which may be because of more thorough collecting. Of these, there are 3 species and 5 subspecies known from other regions, and 5 species and 4 subspecies endemic in the island. Thus, 9 out of 17 are endemic forms, which is more than fifty per cent.

Of the Jamaican endemic forms, some are of particular interest. *Thecla simaethis*, of wide distribution, appears as a subspecies (*jago*) only in Jamaica. Not distantly related to it is the endemic species *Thecla crethona*. This has a close relative in an undescribed species taken on Mount Roraima, Brazil. Both *crethona* and this undescribed species occur at considerable elevations. Mount Roraima is an isolated locality where relict forms might be expected. We suggest that *crethona* and its relative are relict forms, elsewhere eliminated, progenitors perhaps of the *simaethis* strain, and, further, that the subspecies *simaethis jago* is part of the same development, relict in Jamaica.

Another species peculiar to Jamaica (*Leptotes perkinsae*) belongs to

the *cassius* group. Flying with this, is *Leptotes cassius theonus* which is generally distributed in the Greater Antilles. In the *ammon* group, there is *Hemiargus dominica*, which appears to be distinct from any of the *ammon* subspecies, but very closely related. This apparently replaces *ammon* in Jamaica. We have the feeling that both *perkinsae* and *dominica* may be relict forms surviving in Jamaica. Why relict forms, if such these are, should persist in Jamaica, is not immediately apparent, unless it is that Jamaica is somewhat out of the direct island chain.

An area which suggests possibilities is that of the Bahama Islands. These many islands, containing diverse habitats, occupy an area certainly as great as that occupied by Cuba. This region has been little explored, but the recent work of Clench suggests possibilities.

Having become very familiar with these insular forms, we may perhaps attach too great an importance to some of the observations we have made. The inclination to speculate is almost irresistible, but dangerous, because we realize how scanty is our evidence. We have endeavored to summarize the facts and we have indicated what some of them suggest to us, in the hope that our observations may promote discussion. With a further accumulation of material and a better understanding of it, the problems of the distribution of the Antillean Lycaenidae may be more clearly interpreted.

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PLATE 1

All figures are $\times 1.5$

FIGURE 1. *Thecla acis casasi* Comstock and Huntington ♀. Paratype. Santiago de Cuba.

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FIGURE 20. *Hemiargus hanno watsoni* Comstock and Huntington ♂. Paratype. Guayanilla, Puerto Rico, July 22, 1914; collector, F. E. Watson.

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NEW METHODS IN STELLAR DYNAMICS*

By

S. CHANDRASEKHAR †

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* Awarded an A. Cressy Morrison Prize in Natural Science in 1942 by The New York Academy of Sciences. Publication made possible through a grant from the income of the Ralph Winifred Tower Memorial Fund.

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PREFACE

The present paper gives a condensed version of certain new methods which the author has recently been developing for investigating the dynamics of stellar systems. In presenting the subject, it was thought desirable that the emphasis be placed throughout on the physical aspects of the problems and whenever this has required the suppression of the mathematical details I have not avoided doing so. This is particularly true in the more technical parts of the subject.

Since the original version of this paper was submitted to the New York Academy of Sciences in September, 1942, the subject has advanced along several directions. The author is therefore greatly indebted to the Council of the Academy for permission to drastically revise and recast the entire article.

S. C.

July, 1943.

I. THE STATISTICS OF THE GRAVITATIONAL FIELD ARISING FROM A RANDOM DISTRIBUTION OF STARS

The Outline of the Statistical Method

One of the principal problems of stellar dynamics is concerned with the analysis of the nature of the force acting on a star which is a member of a stellar system.¹ In a general way, it is clear that we may broadly distinguish between the influence of the system as a whole and the influence of the immediate neighborhood. The former will be a smoothly varying function of position and time while the latter will be subject to relatively rapid fluctuations (see below).

Considering first the influence of the system as a whole, it appears that we can express it in terms of the gravitational potential $\mathbf{V}(\mathbf{r}; t)$ derived from the function $n(\mathbf{r}, M; t)$ which governs the average spatial distribution of the stars of different masses at time t . Thus

$$\mathbf{V}(\mathbf{r}; t) = -G \int_{-\infty}^{+\infty} \int_0^{\infty} \frac{M n(\mathbf{r}_1, M, t) dM d\mathbf{r}_1}{|\mathbf{r} - \mathbf{r}_1|}, \quad (1)$$

where G denotes the constant of gravitation. The potential $\mathbf{V}(\mathbf{r}; t)$ derived in this manner may be said to represent the "smoothed out"

¹ See for example, Chandrasekhar, S., "Principles of Stellar Dynamics" Chapter II. University of Chicago Press. 1942

distribution of matter in the stellar system. The force per unit mass acting on a star due to the "system as a whole" is therefore given by

$$\mathbf{K} = -\text{grad } \mathbf{V}(\mathbf{r}; t). \quad (2)$$

However, the fluctuations in the *complexion* of the local stellar distribution will make the instantaneous force acting on a star to deviate from the value given by (2). To elucidate the nature and origin of these fluctuations we surround the star under consideration by an element of volume σ which we shall suppose is small enough to contain, on the average, only a relatively few stars. The actual number of stars, which will be found in σ at any given instant of time, will not in general be the average number that will be expected to be in it, namely, $n\sigma$; it will be subject to fluctuations. These fluctuations will naturally be governed by a Poisson distribution with variance $n\sigma$. It is in direct consequence of this changing complexion of the local stellar distribution that the influence of the near neighbors on a star is variable. The average period of such a fluctuation is readily estimated. The order of magnitude of the time involved is evidently that required for two stars to separate by a distance D equal to the average distance between the stars. We may therefore expect that the influence of the immediate neighborhood will fluctuate with an average period of the order of

$$T \simeq \frac{D}{\sqrt{[\mathbf{V}^2]}}, \quad (3)$$

where $[\mathbf{V}^2]^{1/2}$ denotes the root mean square relative velocity between two stars.

In the neighborhood of the sun, $D \sim 3$ parsecs, $[\mathbf{V}^2]^{1/2} \sim 50$ km/sec. Hence,

$$T \text{ (near the sun)} \sim 6 \times 10^4 \text{ years.} \quad (4)$$

When we compare this time with the period of galactic rotation (which is about 2×10^8 years), we observe that, in conformity with our earlier remarks, the fluctuations in the force acting on a star due to the changing local stellar distribution does in fact occur with extreme rapidity compared to the rate at which any of the other physical parameters change. Accordingly, we may write for the force per unit mass acting on a star, the expression

$$\mathbf{F} = \mathbf{K}(\mathbf{r}; t) + \mathbf{F}(t), \quad (5)$$

where \mathbf{K} is derived from the smoothed out distribution, as in equations (1) and (2), and \mathbf{F} denotes the fluctuating force due to the near neighbors. Moreover, if Δt denotes an interval of time, long compared to (3), we may write

$$\mathbf{F}\Delta t = \mathbf{K}\Delta t + \delta\mathbf{u}(t; t+\Delta t), \quad (6)$$

where

$$\delta\mathbf{u}(t; t+\Delta t) = \int_t^{t+\Delta t} \mathbf{F}(\xi) d\xi \quad (\Delta t \gg T). \quad (7)$$

Under the circumstances stated ($\Delta t \gg T$), the accelerations $\delta\mathbf{u}(t; t+\Delta t)$ and $\delta\mathbf{u}(t+\Delta t; t+2\Delta t)$ suffered during two successive intervals $(t, t+\Delta t)$ and $(t+\Delta t, t+2\Delta t)$ will not be expected to show any correlation. We may therefore anticipate the existence of a definite law of distribution which will govern the probability of occurrence of the different values of $\delta\mathbf{u}(t; t+\Delta t)$. We thus see that the acceleration which a star suffers during an interval $\Delta t \gg T$ can be formally expressed as the sum of two terms: a *systematic* term, $\mathbf{K}\Delta t$, due to the action of the gravitational field of the smoothed out distribution and a *stochastic* term, $\delta\mathbf{u}(t; t+\Delta t)$, representing the influence of the near neighbors. Stated in this fashion, we recognize the similarity between our present problems in stellar dynamics with those which occur in the modern theories of Brownian motion.²

We proceed now to the outline of a general method which appears suitable for analyzing the statistical properties of $\mathbf{F}(t)$. The force \mathbf{F} acting on a star, per unit mass, is given by

$$\mathbf{F} = G \sum_i \frac{M_i}{|\mathbf{r}_i|^3} \mathbf{r}_i, \quad (8)$$

where M_i denotes the mass of a typical "field" star and \mathbf{r}_i its position vector relative to the star under consideration; further, in equation (8) the summation is to be extended over all the neighboring stars. The actual value of \mathbf{F} given by equation (8) at any particular instant of time will depend on the instantaneous complexion of the local stellar distribution. It is in consequence subject to fluctuations. We can therefore ask only for the probability of occurrence

$$W(\mathbf{F})dF_x dF_y dF_z = W(\mathbf{F})d\mathbf{F} \quad (9)$$

of \mathbf{F} in the range \mathbf{F} and $\mathbf{F} + d\mathbf{F}$. In evaluating this probability distribution we shall suppose, consistent with the physical situations we have in view, that fluctuations subject only to the restriction of a constant average density occur. However, the specification of $W(\mathbf{F})$ does *not* provide us with all the necessary information concerning the fluctuating force \mathbf{F} . An equally important aspect of the problem concerns the *speed of fluctuations*.

² See a forthcoming article by the author in the "Reviews of Modern Physics."

According to equation (8) the rate of change of F with time is given by

$$f = \frac{dF}{dt} = G \sum_i M_i \left\{ \frac{V_i}{|r_i|^3} - 3r_i \frac{(r_i \cdot V_i)}{|r_i|^5} \right\}, \quad (10)$$

where V_i denotes the velocity of a typical field star *relative* to the star under consideration. It is now apparent that the speed of fluctuations in F can be specified in terms of the bivariate distribution

$$W(F, f), \quad (11)$$

which governs the probability of the simultaneous occurrence of prescribed values for both F and f . It is seen that this distribution function $W(F, f)$ will depend on the assignment of *a priori* probability in the *phase space* in contrast to the distribution $W(F)$ of F , which depends only on a similar assignment in the *configuration space*. While it is possible by an application of a general method, due to Markoff, to write down a general formula for $W(F, f)$, it does not appear feasible to obtain the required distribution function in an explicit form. However, it is possible to obtain explicit formulae for all the first and second moments of f for a given F ; and it appears possible to make some progress in the specification of the statistical properties of F in terms of these moments.

The Statistical Properties of F

We require the stationary distribution of F and its simultaneous rate of change f acting on a given star. Without loss of generality we can suppose that the point under consideration is at the origin, O , of our system of coordinates. About O describe a sphere of radius R and containing N stars. In the first instance we shall suppose that

$$F = G \sum_{i=1}^N \frac{M_i}{|r_i|^3} r_i, \quad (12)$$

and

$$f = G \sum_{i=1}^N M_i \left\{ \frac{V_i}{|r_i|^3} - 3r_i \frac{(r_i \cdot V_i)}{|r_i|^5} \right\}; \quad (13)$$

but we shall later let R and N tend to infinity simultaneously in such a way that

$$\frac{4}{3} \pi R^3 n = N; \quad (R \rightarrow \infty; \quad N \rightarrow \infty; \quad n = \text{constant}). \quad (14)$$

This limiting process is permissible, in view of the fact that the dominant contribution to F is made by the nearest neighbor⁴; consequently, the

³ It is in this respect that the analysis which follows differs from that contained in Chandrasekhar, S., & von Neumann, J. *Astrophysical Jour.* 96: 489, 1942, where the speed of fluctuations in F acting at some fixed point in space is considered.

⁴ Cf. Chandrasekhar, S., *Astrophysical Jour.* 94: 511, 1941 (see particularly § 4).

formal extrapolation to infinity of the density of stars obtained only in a given region of stellar system can hardly affect the results to any appreciable extent.

Using a general method due to Markoff, we can readily write down a general formula for the distribution function $W(F, f)$. We have

$$W(F, f) = \frac{1}{64\pi^6} \int_{|\mathbf{g}|=0}^{\infty} \int_{|\mathbf{\delta}|=0}^{\infty} e^{-i(\mathbf{g} \cdot \mathbf{F} + \mathbf{\delta} \cdot \mathbf{f})} A(\mathbf{g}, \mathbf{\delta}) d\mathbf{g} d\mathbf{\delta}, \quad (15)$$

where

$$A(\mathbf{g}, \mathbf{\delta}) = \text{Limit}_{R \rightarrow \infty} \left[\frac{3}{4\pi R^3} \int_{M=0}^{\infty} \int_{|V|=0}^{\infty} \int_{|r|=0}^R e^{i(\mathbf{g} \cdot \mathbf{\phi} + \mathbf{\delta} \cdot \mathbf{\psi})} \tau dr dV dM \right]^{4\pi R^3 n/3} \quad (16)$$

In equations (15) and (16) \mathbf{g} and $\mathbf{\delta}$ are two auxiliary vectors; n denotes the number of stars per unit volume;

$$\phi = GM \frac{\mathbf{r}}{r^3}; \quad \psi = GM \left(\frac{\mathbf{V}}{r^3} - 3\mathbf{r} \frac{(\mathbf{r} \cdot \mathbf{V})}{r^5} \right). \quad (17)$$

Further,

$$\tau dV dM = \tau(V; M) dV dM \quad (18)$$

gives the probability that a star with a relative velocity in the range $(V, V + dV)$ and with a mass between M and $M + dM$ will be found. It should also be noted that in writing equations (15) and (16) we have supposed that the fluctuations in the local stellar distribution which occur are subject only to the restriction of a constant average density.

Since

$$\frac{3}{4\pi R^3} \int_{M=0}^{\infty} \int_{|r|=0}^R \int_{|V|=0}^{\infty} \tau dV dr dM = 1, \quad (19)$$

we can rewrite (16) as

$$A(\mathbf{g}, \mathbf{\delta}) = \text{Limit}_{R \rightarrow \infty} \left[1 - \frac{3}{4\pi R^3} \int_{M=0}^{\infty} \int_{|r|=0}^R \int_{|V|=0}^{\infty} [1 - e^{i(\mathbf{g} \cdot \mathbf{\phi} + \mathbf{\delta} \cdot \mathbf{\psi})}] \tau dV dr dM \right]^{4\pi R^3 n/3} \quad (20)$$

The integral over \mathbf{r} which occurs in equation (20) is seen to be absolutely convergent when extended over all \mathbf{r} , i.e., also for $r \rightarrow \infty$. Hence, we can write

$$A(\varrho, \delta) = \lim_{R \rightarrow \infty} \left[1 - \frac{3}{4\pi R^3} \int_{|r|=0}^{\infty} \int_{|\tau|=0}^{\infty} \int_{|V|=0}^{\infty} [1 - e^{i(\varrho \cdot \phi + \delta \cdot \psi)}] \tau dV dr dM \right]^{4\pi R^3 n/3} \quad (21)$$

or

$$A(\varrho, \delta) = e^{-n \ell(\varrho, \delta)}, \quad (22)$$

where

$$C(\varrho, \delta) = \int_0^{\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} [1 - e^{i(\varrho \cdot \phi + \delta \cdot \psi)}] \tau dr dV dM. \quad (23)$$

This formally solves the problem. It does not, however, appear that the integral representing $C(\varrho, \delta)$ can be evaluated explicitly in terms of any of the known functions. But if we are interested only in the distribution $W(F)$ of F and in the moments of f for a given F then we need only the behavior of $A(\varrho, \delta)$, and therefore also of $C(\varrho, \delta)$, for $|\delta| \rightarrow 0$, for the distribution $W(F)$ is clearly given by

$$W(F) = \int_{-\infty}^{+\infty} W(F, f) df. \quad (24)$$

Similarly, the first and the second moments of the components f_{ξ}, f_{η} , and f_{ζ} of f along three directions ξ, η and ζ at right angles to each other are given by

$$W(F) \bar{f}_{\mu} = \int_{-\infty}^{+\infty} W(F, f) f_{\mu} df \quad (\mu = \xi, \eta, \zeta), \quad (25)$$

and

$$W(F) \bar{f}_{\mu} \bar{f}_{\nu} = \int_{-\infty}^{+\infty} W(F, f) f_{\mu} f_{\nu} df \quad (\mu, \nu = \xi, \eta, \zeta). \quad (26)$$

Substituting now for $W(F, f)$ according to equation (15) in the foregoing equations we obtain

$$\left. \begin{aligned} W(F) &= \frac{1}{64\pi^6} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} e^{-i(\varrho \cdot F + \delta \cdot f)} A(\varrho, \delta) df d\varrho d\delta, \\ W(F) \bar{f}_{\mu} &= \frac{1}{64\pi^6} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} e^{-i(\varrho \cdot F + \delta \cdot f)} A(\varrho, \delta) f_{\mu} df d\varrho d\delta, \\ W(F) \bar{f}_{\mu} \bar{f}_{\nu} &= \frac{1}{64\pi^6} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} e^{-i(\varrho \cdot F + \delta \cdot f)} A(\varrho, \delta) f_{\mu} f_{\nu} df d\varrho d\delta. \end{aligned} \right\} \quad (27)$$

But

$$\left. \begin{aligned} \frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\sigma \cdot f} df &= \delta(\sigma_x) \delta(\sigma_y) \delta(\sigma_z), \\ \frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\sigma \cdot f} f_{\xi} df &= i\delta'(\sigma_x) \delta(\sigma_y) \delta(\sigma_z), \\ \frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\sigma \cdot f} f_{\xi}^2 df &= -\delta''(\sigma_x) \delta(\sigma_y) \delta(\sigma_z), \\ \frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\sigma \cdot f} f_{\xi} f_{\eta} df &= -\delta'(\sigma_x) \delta'(\sigma_y) \delta(\sigma_z), \end{aligned} \right\} \quad (28)$$

etc. In equations (28), δ denotes Dirac's δ -function and δ' and δ'' its first and second derivatives; and remembering also that

$$\left. \begin{aligned} \int_{-\infty}^{+\infty} f(x) \delta(x) dx &= f(0); & \int_{-\infty}^{+\infty} f(x) \delta'(x) dx &= -f'(0); \\ \int_{-\infty}^{+\infty} f(x) \delta''(x) dx &= f''(0), \end{aligned} \right\} \quad (29)$$

equations (27) reduce to

$$W(F) = \frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\sigma \cdot F} [A(\sigma, \sigma)]_{|\sigma|=0} d\sigma, \quad (30)$$

$$W(F) \overline{f_{\mu}} = -\frac{i}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\sigma \cdot F} \left[\frac{\partial}{\partial \sigma_{\mu}} A(\sigma, \sigma) \right]_{|\sigma|=0} d\sigma, \quad (31)$$

and

$$W(F) \overline{f_{\mu} f_{\nu}} = -\frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\sigma \cdot F} \left[\frac{\partial^2}{\partial \sigma_{\mu} \partial \sigma_{\nu}} A(\sigma, \sigma) \right]_{|\sigma|=0} d\sigma. \quad (32)$$

We accordingly see that the distribution function $W(F)$ and all the first and the second moments of f for a given F can be evaluated from a series expansion of $A(\sigma, \sigma)$ [or of $C(\sigma, \sigma)$] which is correct up to the second order in $|\sigma|$. The development of such a series is long and tedious.

Omitting, therefore, all the details of the calculations, we give only the final result. It is found that

$$C(\varrho, \sigma) = \frac{4}{15} (2\pi)^{1/2} \langle \bar{M}^{3/2} \rangle |\varrho|^{3/2} \left\{ \begin{aligned} &+ \frac{2}{3} \pi i (\sigma_1 \bar{M} \bar{V}_1 + \sigma_2 \bar{M} \bar{V}_2 - 2\sigma_3 \bar{M} V_3) \\ &+ \frac{3}{28} (2\pi)^{3/2} \langle \bar{V}^{1/2} \rangle |\varrho|^{-3/2} [(5\sigma_1^2 + 4\sigma_2^2 - 2\sigma_3^2) \bar{M}^{1/2} \bar{V}_1^2 \\ &+ (4\sigma_1^2 + 5\sigma_2^2 - 2\sigma_3^2) \bar{M}^{1/2} \bar{V}_2^2 \\ &+ (4\sigma_3^2 - 2\sigma_1^2 - 2\sigma_2^2) \bar{M}^{1/2} \bar{V}_3^2 - 8\sigma_2 \sigma_3 \bar{M}^{1/2} V_2 V_3 \\ &- 8\sigma_1 \sigma_3 \bar{M}^{1/2} \bar{V}_1 \bar{V}_3 + 2\sigma_1 \sigma_2 \bar{M}^{1/2} \bar{V}_1 \bar{V}_2] + O(|\sigma|^3), \quad (|\sigma| \rightarrow 0), \end{aligned} \right\} \quad (33)$$

where a bar indicates that the corresponding quantity has been averaged with the weight function $\tau(V; M)$ (see equation [18]); further, in equation (33), $(\sigma_1, \sigma_2, \sigma_3)$ and (V_1, V_2, V_3) are the components of σ and V in a system of coordinates in which the Z -axis is in the direction of ϱ .

In equation (33) $V = (V_1, V_2, V_3)$ denotes of course the velocity of a field star relative to the one under consideration. If we now let u and v denote respectively the velocities of the field star and the star under consideration in an appropriately chosen local standard of rest, then

$$V = u - v. \quad (34)$$

In our further discussion we shall introduce the assumption that the distribution of the velocities u among the stars is *spherical*; i.e., the distribution function $\Psi(u)$ has the form

$$\Psi(u) \equiv \Psi[j^2(M)|u|^2], \quad (35)$$

where Ψ is an arbitrary function of the argument specified and the parameter j (of the dimensions of $[\text{velocity}]^{-1}$) can be a function of the mass of the star. This assumption for the distribution of the peculiar velocities u implies that the probability function $\tau(V; M)$ must be expressible as

$$\tau(V; M) = \Psi[j^2(M)|u|^2] \chi(M) \quad (36)$$

where $\chi(M)$ governs the distribution over the different masses. For a function τ of this form we clearly have

$$\left. \begin{aligned} \overline{MV}_i &= -\bar{M}v_i; & \overline{M^{1/2}V_i^2} &= \frac{1}{3} \overline{M^{1/2}|u|^2} + \bar{M}^{1/2}v_i^2 \quad (i = 1, 2, 3) \\ \overline{M^{1/2}V_i V_j} &= \bar{M}^{1/2}v_i v_j, & [i, j &= 1, 2, 3; \quad i \neq j]. \end{aligned} \right\} \quad (37)$$

Substituting these values in equation (33) we find, after some minor reductions, that

$$\begin{aligned}
 U(\mathbf{q}, \sigma) = & \left. \begin{aligned}
 & \frac{1}{15} (2\pi)^{3/2} \overline{M}^{3/2} \mathbf{q}^{-3/2} - \frac{2}{3} \pi i \overline{M} (\sigma_1 v_1 + \sigma_2 v_2 - 2\sigma_3 v_3) \\
 & + \frac{1}{4} (2\pi)^{3/2} G^{1/2} \overline{M}^{1/2} |\mathbf{u}|^2 |\mathbf{q}|^{-3/2} (\sigma_1^2 + \sigma_2^2) \\
 & + \frac{3}{28} (2\pi)^{3/2} G^{1/2} \overline{M}^{1/2} |\mathbf{q}|^{-3/2} \{ \sigma_1^2 (5v_1^2 + 4v_2^2 - 2v_3^2) \\
 & + \sigma_2^2 (4v_1^2 + 5v_2^2 - 2v_3^2) + \sigma_3^2 (4v_3^2 - 2v_1^2 - 2v_2^2) \\
 & - 8\sigma_2\sigma_3 v_1 v_3 - 8\sigma_3\sigma_1 v_3 v_1 + 2\sigma_1\sigma_2 v_1 v_2 \} + O(|\mathbf{q}|^3), \quad (|\mathbf{q}| \rightarrow 0).
 \end{aligned} \right\} \quad (38)
 \end{aligned}$$

With a series expansion of this form, we can, as we have already remarked, evaluate the distribution $W(F)$ and all the first and the second moments of \mathbf{f} for a given F .

THE DISTRIBUTION $W(F)$

According to equations (30) and (38) we have

$$W(F) = \frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\mathbf{q} \cdot \mathbf{F} - a|\mathbf{q}|^2} d\mathbf{q}, \quad (39)$$

where we have written

$$a = \frac{1}{15} (2\pi G)^{3/2} \overline{M}^{3/2} n. \quad (40)$$

From equation (39) we derive the formula⁶

$$W(F) = \frac{1}{4\pi a^2} \frac{H(\beta)}{\beta^2}, \quad (41)$$

where

$$H(\beta) = \frac{2}{\pi\beta} \int_0^\infty e^{-(x|\beta|)^{3/2}} x \sin x \, dx, \quad (42)$$

and β measures $|\mathbf{F}|$ in units of Q_H where

$$Q_H = a^{2/3} = 2.6031 (G^{1/2} \overline{M}^{3/2} n)^{2/3}. \quad (43)$$

The function $H(\beta)$ has been numerically evaluated and tabulated in Chandrasekhar and von Neumann's paper.

THE FIRST MOMENT OF \mathbf{f} : DYNAMICAL FRICTION

Turning next to the first moment of \mathbf{f} it is found after some lengthy calculations that

$$\bar{\mathbf{f}} = \overline{\left(\frac{d\mathbf{F}}{dt} \right)}_{\mathbf{F}, \nu} = -\frac{2}{3} \pi G \overline{M} n B \left(\frac{|\mathbf{F}|}{Q_H} \right) \left(\nu - 3 \frac{\nu \cdot \mathbf{F}}{F^2} \mathbf{F} \right), \quad (44)$$

where Q_H is the normal field defined in equation (43) and

⁶ Cf. Chandrasekhar, S., & von Neumann, J. *Astrophysical Jour.* **95**: 489, 1942. (§ 7).

$$B(\beta) = 3 \frac{\int_0^\beta H(\beta) d\beta}{\beta H(\beta)} - 1. \quad (45)$$

The function $B(\beta)$ has the following asymptotic forms:

$$\left. \begin{aligned} B(\beta) &\rightarrow \frac{1}{15} \Gamma\left(\frac{10}{3}\right) \beta^2 & (\beta \rightarrow 0), \\ B(\beta) &\rightarrow \frac{8}{5} \sqrt{\frac{\pi}{2}} \beta^{3/2} & (\beta \rightarrow \infty). \end{aligned} \right\} \quad (46)$$

We shall first examine certain formal consequences of equation (44).

Multiplying equation (44) scalarly with \mathbf{F} we obtain

$$\mathbf{F} \cdot \left(\frac{d\mathbf{F}}{dt} \right)_{\mathbf{F}, \mathbf{v}} = \frac{4}{3} \pi G \bar{M} n B \left(\frac{|\mathbf{F}|}{Q_H} \right) (\mathbf{v} \cdot \mathbf{F}) \quad (47)$$

but

$$\mathbf{F} \cdot \left(\frac{d\mathbf{F}}{dt} \right)_{\mathbf{F}, \mathbf{v}} = |\mathbf{F}| \left(\frac{d|\mathbf{F}|}{dt} \right)_{\mathbf{F}, \mathbf{v}}. \quad (48)$$

Hence,

$$\left(\frac{d|\mathbf{F}|}{dt} \right)_{\mathbf{F}, \mathbf{v}} = \frac{4}{3} \pi G \bar{M} n B \left(\frac{|\mathbf{F}|}{Q_H} \right) \frac{\mathbf{v} \cdot \mathbf{F}}{|\mathbf{F}|}. \quad (49)$$

On the other hand, if F_j denotes the component of \mathbf{F} in an arbitrary direction at right angles to the direction of \mathbf{v} then, according to equation (44),

$$\left(\frac{dF_j}{dt} \right)_{\mathbf{F}, \mathbf{v}} = 2 \pi G \bar{M} n B \left(\frac{|\mathbf{F}|}{Q_H} \right) \frac{\mathbf{v} \cdot \mathbf{F}}{|\mathbf{F}|^2} F_j. \quad (50)$$

Combining equations (49) and (50) we have

$$\frac{1}{F_j} \left(\frac{dF_j}{dt} \right)_{\mathbf{F}, \mathbf{v}} = \frac{3}{2} \frac{1}{|\mathbf{F}|} \left(\frac{d|\mathbf{F}|}{dt} \right)_{\mathbf{F}, \mathbf{v}}. \quad (51)$$

Equation (51) is clearly equivalent to

$$\left[\frac{d}{dt} \left(\log F_j - \frac{3}{2} \log |\mathbf{F}| \right) \right]_{\mathbf{F}, \mathbf{v}} = 0. \quad (52)$$

We have thus proved that

$$\left[\frac{d}{dt} \left(\frac{F_j}{|\mathbf{F}|^{3/2}} \right) \right]_{\mathbf{F}, \mathbf{v}} = 0. \quad (53)$$

We shall now examine the physical consequences of equation (44) more closely. In words, the meaning of this equation is that the component of

$$-\frac{2}{3} \pi G M n B \left(\frac{|\mathbf{F}|}{Q_H} \right) \left(\nu - 3 \frac{\nu \cdot \mathbf{F}}{|\mathbf{F}|^2} \mathbf{F} \right) \quad (54)$$

along any particular direction gives the average value of the rate of change in the force \mathbf{F} per unit mass acting on a star that is to be expected in the specified direction, when the star is moving with a velocity ν in an appropriately chosen local standard of rest. Stated in this manner, we at once see the essential difference in the stochastic variations of \mathbf{F} with time in the two cases, $\nu = 0$ and $|\nu| \neq 0$. In the former case, $\overline{\dot{\mathbf{F}}} \equiv 0$; but this is not generally true when $\nu \neq 0$. Or expressed differently, when $\nu = 0$ the changes in \mathbf{F} occur with equal probability in all directions, while this is not the case when $\nu \neq 0$. The true nature of this difference is brought out very clearly when we consider

$$\overline{\left(\frac{d|\mathbf{F}|}{dt} \right)}_{\mathbf{F}, \nu} \quad (55)$$

according to equation (49). Remembering that $B(\beta) \geq 0$ for $\beta \geq 0$, we conclude from equation (49) that

$$\overline{\left(\frac{d|\mathbf{F}|}{dt} \right)}_{\mathbf{F}, \nu} > 0 \quad \text{if} \quad \nu \cdot \mathbf{F} > 0. \quad (56)$$

and

$$\overline{\left(\frac{d|\mathbf{F}|}{dt} \right)}_{\mathbf{F}, \nu} < 0 \quad \text{if} \quad \nu \cdot \mathbf{F} < 0. \quad (57)$$

In other words, if \mathbf{F} has a positive component in the direction of ν , \mathbf{F} increases on the average, while if \mathbf{F} has a negative component in the direction of ν , \mathbf{F} decreases on the average. This essential asymmetry introduced by the direction of ν may be expected to give rise to the phenomenon of dynamical friction.

The characteristic aspects of the situation governed by equation (44) are best understood when we contrast it with the case $\nu = 0$. Under these circumstances, we can visualize the motion of the representative point in the velocity space somewhat as follows.⁷ The representative point suffers small random displacements in a manner that can be adequately described by the problem of random flights or more generally as Brownian motion. More specifically, the star may be assumed to suffer a large number of discrete increases in velocity of amounts $T(\mathbf{F})\mathbf{F}$, where T denotes the mean life of the state \mathbf{F} (see subsection below). Moreover, these increases may be assumed to take place in

⁷ Cf. Chandrasekhar, S., *Astrophysical Jour.* **94**: 511. 1941. (§§ 2 and 7.)

random directions. Accordingly, we may conclude that the mean square increase $|\Delta \mathbf{v}|^2$ in the velocity to be expected in time t is given by

$$|\Delta \mathbf{v}|^2 = |\mathbf{F}|^2 T t. \quad (58)$$

An alternative way of describing the same situation is that if we denote by $W(\mathbf{v}; t)$ the probability that the star has a velocity \mathbf{v} at time t when the velocity at $t = 0$ is \mathbf{v}_0 , then W satisfies the *diffusion equation*

$$\frac{\partial W}{\partial t} = q \left(\frac{\partial^2 W}{\partial v_1^2} + \frac{\partial^2 W}{\partial v_2^2} + \frac{\partial^2 W}{\partial v_3^2} \right). \quad (59)$$

with the "coefficient of diffusion" q having the value

$$q = \frac{1}{6} \overline{|\mathbf{F}|^2 T}. \quad (60)$$

The solution of equation (59) for our purposes then is

$$W(\mathbf{v}, t; \mathbf{v}_0) = \frac{1}{(4\pi q t)^{3/2}} e^{-|\mathbf{v} - \mathbf{v}_0|^2 / 4 q t}. \quad (61)$$

The formula (58) is seen to be an immediate consequence of the solution (61).

Returning to the discussion of the case governed by equations (44) and (49), it is at once clear that the idealization of the motion of the representative point in the velocity space, as a problem in random flights, can no longer be valid. For, according to (56) and (57), during a given state of fluctuation of \mathbf{F} a star is likely to suffer a greater absolute amount of acceleration if $(\mathbf{v} \cdot \mathbf{F})$ is negative than if $(\mathbf{v} \cdot \mathbf{F})$ is positive. But the *a priori* probability for $(\mathbf{v} \cdot \mathbf{F})$ to be positive or negative is equal. Hence, when integrated over a large number of fluctuations the star must suffer cumulatively a larger absolute amount of acceleration in a direction opposite to its own direction of motion than in the direction of motion. In other words we may expect a net tendency for the star to be relatively decelerated in the direction of its motion; further, this tendency is proportional to $|\mathbf{v}|$. But these are exactly what are implied by the existence of dynamical friction. (See Part II where the question of dynamical friction is considered in greater detail.)

THE SECOND MOMENT OF $[f]$ AND THE MEAN LIFE OF THE STATE $[F]$

According to equation (32)

$$W(F) \overline{f_1^2} = -\frac{1}{8\pi^3} \int_{-\infty}^{+\infty} e^{-i\mathbf{q} \cdot \mathbf{F}} [\nabla^2 A(\mathbf{q}, \mathbf{q})]_{q_1=0} d\mathbf{q}. \quad (62)$$

Using the expansion (33) for $C(\mathbf{g}, \mathbf{g})$ we find after some lengthy calculations that

$$\overline{|\mathbf{f}|^2}_{\mathbf{F}, \mathbf{v}} = 2ab \left\{ \frac{\beta^{1/2}}{H(\beta)} \left\{ 2G(\beta) + 7k[\sin^2 \alpha G(\beta) - (3 \sin^2 \alpha - 2)I(\beta)] \right\} \right. \\ \left. + \frac{g^2}{\beta H(\beta)} \left\{ (4 - 3 \sin^2 \alpha) \beta H(\beta) + 3(3 \sin^2 \alpha - 2)K(\beta) \right\} \right\} \quad (63)$$

where α denotes the angle between the directions of \mathbf{F} and \mathbf{v} ,

$$\left. \begin{aligned} a &= \frac{4}{15} (2\pi G)^{3/2} \overline{M}^{3/2} n, & b &= \frac{1}{4} (2\pi)^{3/2} G^{1/2} \overline{M}^{1/2} \overline{\mathbf{u}}^2 n, \\ g &= \frac{2}{3} \pi G \overline{M} |\mathbf{v}| n, & k &= \frac{3}{7} \frac{\overline{M}^{1/2} \mathbf{v}^2}{\overline{M}^{1/2} \overline{\mathbf{u}}^2} \end{aligned} \right\} \quad (64)$$

and

$$\left. \begin{aligned} H(\beta) &= \frac{2}{\pi \beta} \int_0^\pi e^{-\frac{1}{2} \beta \sin^2 x} x \sin x \, dx, \\ G(\beta) &= \frac{3}{2} \int_0^\beta \beta^{-3/2} H(\beta) d\beta, \\ I(\beta) &= \beta^{-3/2} \int_0^\beta \beta^{1/2} G(\beta) d\beta, \\ K(\beta) &= \int_0^\beta H(\beta) d\beta. \end{aligned} \right\} \quad (65)$$

Averaging equation (63) for all possible mutual orientations of the two vectors \mathbf{F} and \mathbf{v} we readily find that

$$\overline{|\mathbf{f}|^2}_{|\mathbf{F}|, |\mathbf{v}|} = 4ab \left\{ \frac{\beta^{1/2} G(\beta)}{H(\beta)} \left(1 + \frac{7}{3} k \right) + \frac{g^2}{2ab} \right\}, \quad (66)$$

or substituting for k and $g^2/2ab$ from (64), we find that

$$\overline{|\mathbf{f}|^2}_{|\mathbf{F}|, |\mathbf{v}|} = 4ab \left\{ \frac{\beta^{1/2} G(\beta)}{H(\beta)} \left(1 + \frac{\overline{M}^{1/2} |\mathbf{v}|^2}{\overline{M}^{1/2} \overline{\mathbf{u}}^2} \right) + \frac{5}{12\pi} \frac{\overline{M}^2 \mathbf{v}^2}{\overline{M}^{3/2} \overline{M}^{1/2} \overline{\mathbf{u}}^2} \right\}. \quad (67)$$

In terms of equation (67) we can define an approximate formula for the mean life of the state $|\mathbf{F}|$ according to³

$$T_{|\mathbf{F}|, |\mathbf{v}|} = \frac{|\mathbf{F}|}{\sqrt{\overline{|\mathbf{f}|^2}_{|\mathbf{F}|, |\mathbf{v}|}}}. \quad (68)$$

³ Cf. Chandrasekhar, S., & von Neumann, J. *Astrophysical Jour.* **95**: 489. 1942. Equation (167).

Combining equations (67) and (68) we find that

$$T_{F, \mathbf{v}} = T_{F, 0} \left[1 + \frac{\overline{M^{1/2} \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} + \frac{5}{12\pi} \frac{\overline{M^2 \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} \frac{\overline{H(\beta)}}{\beta^{1/2} G(\beta)} \right]^{1/2}, \quad (69)$$

where $T_{F, 0}$ denotes the mean life when $\mathbf{v} = 0$:

$$T_{F, 0} = \sqrt{\frac{\overline{a^{1/3}}}{4b}} \frac{\overline{\beta^3 H(\beta)}}{G(\beta)}. \quad (70)$$

Equation (70) suggests that we measure T in terms of the following unit, t_0 , which appears natural to this problem:

$$t_0 = \sqrt{\frac{\overline{a^{1/3}}}{4b}}. \quad (71)$$

Substituting for a and b from equation (64), we find that

$$t_0 = \left. \begin{aligned} & \frac{1}{(30)^{1/6} \pi^{1/2}} \left(\frac{[\overline{M^3}]^{1/3}}{[\overline{M^{1/2} \mathbf{u}^2}]} \right)^{1/2} \frac{1}{n^{1/3}} \\ & = \frac{0.3201}{n^{1/3}} \left(\frac{[\overline{M^3}]^{1/3}}{[\overline{M^{1/2} \mathbf{u}^2}]} \right)^{1/2} \end{aligned} \right\} \quad (72)$$

And, finally, if we denote by $\tau(\beta; \mathbf{v})$ the mean life expressed in this unit, we have

$$\tau(\beta; \mathbf{v}) = \tau(\beta; 0) \left[1 + \frac{\overline{M^{1/2} \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} + \frac{5}{12\pi} \frac{\overline{M^2 \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} \frac{\overline{H(\beta)}}{\beta^{1/2} G(\beta)} \right]^{1/2}. \quad (73)$$

From equation (73) we derive the asymptotic formulae

$$\tau \rightarrow 3 \frac{1}{\left[1 + \frac{\overline{M^{1/2} \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} + \frac{5}{12\pi} \frac{\overline{M^2 \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} \right]^{1/2}} \quad (\beta \rightarrow 0), \quad (74)$$

and

$$\tau \sim \sqrt{\frac{15}{8}} \frac{1}{\left[1 + \frac{\overline{M^{1/2} \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} \right]^{1/2}} \beta^{-1/2} \quad (\beta \rightarrow \infty). \quad (75)$$

The function $\tau(\beta; 0)$ is tabulated in Chandrasekhar and von Neumann's paper. Our present results show that approximately

$$\tau(\beta; \mathbf{v}) \sim \tau(\beta; 0) \frac{1}{\left[1 + \frac{\overline{M^{1/2} \mathbf{v}^2}}{\overline{M^{1/2} \mathbf{u}^2}} \right]^{1/2}}. \quad (76)$$

According to equations (74) and (75), the approximate formula (76) may

be expected to give values of τ correct to within 15 per cent over the entire range of β .

We may particularly draw attention to the very short lives of the weak fields.

II. DYNAMICAL FRICTION AND THE PRINCIPLES OF STATISTICAL DYNAMICS

General Considerations

As we have seen in Part I, in a first approximative discussion of the fluctuating part of the gravitational field acting on a star, we may suppose that the probability function $W(\mathbf{u}, t)$ governing the occurrence of the velocity \mathbf{u} at time t satisfies the diffusion equation (see equation [59]).

$$\frac{\partial W}{\partial t} = q \nabla_{\mathbf{u}}^2 W. \quad (77)$$

According to this equation, the probability distribution of the velocities \mathbf{u} at time t when it is known with certainty that the star had the velocity \mathbf{u}_0 at time $t = 0$ is given by

$$W(\mathbf{u}, t; \mathbf{u}_0) = \frac{1}{(4\pi q t)^{3/2}} e^{-\frac{1}{2}(\mathbf{u} - \mathbf{u}_0)^2 / q t}. \quad (78)$$

We shall now indicate why the considerations outlined above cannot be valid for times which are long compared to \mathbf{u}^2 / q where \mathbf{u}^2 denotes the mean square velocity of the stars in an appropriately chosen local standard of rest. For, if $W(\mathbf{u}, t; \mathbf{u}_0)$ according to equation (78) were valid for all times, then the probability that a star may have suffered any arbitrarily assigned large acceleration can be made as close to unity as we may choose by letting t approach infinity. This is, however, contrary to what we should expect on general grounds, namely, that $W(\mathbf{u}, t; \mathbf{u}_0)$ approaches a Maxwellian distribution independently of \mathbf{u}_0 as $t \rightarrow \infty$. Expressed somewhat differently, we should strictly suppose that the stochastic variations in the velocity which a star experiences must be such as to leave an initial Maxwellian distribution of the velocities invariant. This is evidently not the case with the process described by equation (77). And the question now arises as to how we can generalize our earlier approximate considerations leading to equation (77) so that the underlying stochastic process may satisfy the criterion stated above. We shall now show how this can be achieved by the introduction of *dynamical friction*. More specifically, we shall suppose that the acceleration $\Delta \mathbf{u}$ which a star experiences in an interval of time Δt (long compared to the periods of the elementary fluctuations in \mathbf{F} but short compared to

the intervals during which \mathbf{u} may be expected to change appreciably) can be expressed as the sum of two terms in the form

$$\Delta \mathbf{u} = \delta \mathbf{u}(\Delta t) - \eta \mathbf{u} \Delta t \quad (79)$$

where the first term on the right-hand side is governed by the probability distribution

$$\psi(\delta \mathbf{u}[\Delta t]) = \frac{1}{(4\pi q \Delta t)^{3/2}} e^{-|\delta \mathbf{u} - \text{grad}_{\mathbf{u}} q \Delta t|^2 / 4q \Delta t}, \quad (80)$$

and where the second term $-\eta \mathbf{u} \Delta t$ represents a *deceleration* of the star in the direction of its motion by an amount depending on $|\mathbf{u}|$. The constant of proportionality η can therefore be properly called the *coefficient of dynamical friction*.

With the underlying stochastic process defined as in equations (79) and (80), the probability distribution $W(\mathbf{u}, t + \Delta t)$ of \mathbf{u} at time $t + \Delta t$ can be derived from the distribution $W(\mathbf{u}, t)$ at the earlier time t by means of the integral equation

$$W(\mathbf{u}, t + \Delta t) = \int_{-\infty}^{+\infty} W(\mathbf{u} - \Delta \mathbf{u}, t) \psi(\mathbf{u} - \Delta \mathbf{u}; \Delta \mathbf{u}) d(\Delta \mathbf{u}), \quad (81)$$

where $\psi(\mathbf{u}; \Delta \mathbf{u})$ denotes the transition probability (see equation [80])

$$\psi(\mathbf{u}; \Delta \mathbf{u}) = \frac{1}{(4\pi q \Delta t)^{3/2}} e^{-|\Delta \mathbf{u} - \text{grad}_{\mathbf{u}} q \Delta t + \eta \mathbf{u} \Delta t|^2 / 4q \Delta t} \quad (82)$$

Expanding $W(\mathbf{u}, t + \Delta t)$, $W(\mathbf{u} - \Delta \mathbf{u}, t)$ and $\psi(\mathbf{u} - \Delta \mathbf{u}; \Delta \mathbf{u})$ which occur in equation (81) in the form of Taylor series, evaluating the various moments of $\Delta \mathbf{u}$ according to equation (82) and passing finally to the limit we obtain the following equation

$$\frac{\partial W}{\partial t} = \text{div}_{\mathbf{u}} (q \text{grad}_{\mathbf{u}} W + \eta W \mathbf{u}). \quad (83)$$

Finally, the condition that the Maxwellian distribution

$$\left(\frac{3}{2\pi |\mathbf{u}|^2} \right)^{3/2} e^{-3|\mathbf{u}|^2/2|\bar{\mathbf{u}}|^2} \quad (84)$$

satisfy equation (83) *identically* requires that q and η be related according to

$$q = \frac{1}{3} |\bar{\mathbf{u}}|^2 \eta. \quad (85)$$

Summarizing the conclusion reached, we may say that *general considerations, such as the invariance of the Maxwellian distribution to the underlying stochastic process, require that stars experience dynamical friction during their motion.*

The Resolution of Certain Fallacies and an Elementary Derivation of the Coefficient of Dynamical Friction

The conclusion we have reached in the preceding paragraph appears contrary to what might be expected on first sight. For we *might* argue in the following manner:

(a) Suppose we consider a star with a velocity $|u|$ appreciably less than the root mean square velocity $(\overline{|u|^2})^{1/2}$. We would then expect it to encounter oftener stars with velocities greater than its own than stars with velocities less than its own. And, consequently, we might be led to believe that stars with velocities less than the average would be systematically accelerated and, similarly, that stars with velocities greater than the average would be systematically decelerated.

How then does dynamical friction come to operate on *all* stars? Before we answer this question we shall state the second paradox.

(b) We might go farther and even argue that the conclusion reached in (a) is "reasonable." For, it might be supposed that systematically different effects on stars with relatively large, respectively small velocities, are required for the statistical maintenance of the average (i.e., normal) conditions.

In view of the great importance of dynamical friction for statistical dynamics, we shall analyze the questions raised above in some detail and expose the fallacies involved in (a) and (b).

First, it is easy to show that (b) is a plain misunderstanding. For, there is nothing obvious in the requirement that for the statistical maintenance of the average conditions stars differing from the average conditions should be affected differently according to the *sense* of their departure from the normal state. Indeed, the requirement that the normal conditions are self perpetuating is to state in a different form one of two things: Either, that starting from any arbitrary initial state we approach the normal state (e.g., the Maxwellian distribution of the velocities) as $t \rightarrow \infty$; or, that once the normal state has been attained it continues to be maintained. It is now apparent that these conditions can be met only if a given star behaves at later times in a manner less and less dependent on an initial state as time goes on; or expressing the same thing somewhat differently, we should much rather expect a star to gradually lose all trace of its initial state as the time progresses. Such a gradual loss of "memory" can be achieved only by the operation of a dissipative force like dynamical friction which will gradually damp out any given initial velocity. Thus, if we assume for the sake of simplicity, that η is independent of $|u|$, then the *average* velocity at later times will tend to zero according as

$$\bar{\mathbf{u}} = \mathbf{u}_0 e^{-\eta t}. \quad (86)$$

But this is not to imply that the mean square velocity tends to zero. Indeed, the restoration of a Maxwellian distribution of velocities from an arbitrary initial state requires that

$$\bar{\mathbf{u}} \rightarrow 0 \quad \text{and} \quad |\bar{\mathbf{u}}|^2 \rightarrow \text{a constant as } t \rightarrow \infty. \quad (87)$$

To achieve the first of the two foregoing conditions we need dynamical friction. Thus, the conclusions reached in (a), if valid, are contrary to the requirement for the restoration and maintenance of the normal state. It is therefore necessary to show wherein the argumentation of (a) is in error, and this we now proceed to do.

The way to refute arguments such as (a) is, of course, to actually verify directly whether or not a star with a given initial velocity is decelerated on the average independent of the magnitude of its velocity. For this purpose, it is perhaps simplest and most instructive to examine the problem on an approximation in which the fluctuations in \mathbf{F} are analyzed in terms of single encounters each idealized as a two-body problem. On this approximation the increments in velocity Δu_{\parallel} and Δu_{\perp} which a star with a velocity $u = |\mathbf{u}|$ and mass m suffers as the result of an encounter with another star, in directions which are respectively parallel to and perpendicular to the direction of motion, can be specified in terms of the parameters defining the encounter. We have⁹

$$\Delta u_{\parallel} = -\frac{2m_1}{m_1 + m} [(u - v_1 \cos \theta) \cos \psi + v_1 \sin \theta \cos \Theta \sin \psi] \cos \psi, \quad (88)$$

$$\Delta u_{\perp} = \left. \begin{aligned} &\pm \frac{2m_1}{m_1 + m} [v_1^2 + u^2 - 2uv_1 \cos \theta \\ &- \{(u - v_1 \cos \theta) \cos \psi + v_1 \sin \theta \cos \Theta \sin \psi\}^2]^{1/2} \cos \psi \end{aligned} \right\} \quad (89)$$

where m_1 and v_1 denote the mass and velocity of a typical field star and the rest of the symbols have the same meanings as in "Stellar Dynamics," Chapter II (see particularly, pp. 51-64).

According to equation (89), and as can indeed be expected on general symmetry grounds, Δu_{\perp} when summed over a large number of encounters vanishes identically. But this is not the case with Δu_{\parallel} , for the net increase in the velocity which the star suffers in the direction of its motion during a time Δt (long compared to the periods of the elementary fluctuations in \mathbf{F} , but short compared to the time intervals during which $|\mathbf{u}|$ may be expected to change appreciably) is given by

⁹ Cf. Chandrasekhar, S., "Principles of Stellar Dynamics," p. 229 (equation 5.721). University of Chicago Press. 1942 This monograph will be referred to hereafter as "Stellar Dynamics."

$$\Sigma \Delta u_{||} = \Delta t \int_0^\infty dv_1 \int_0^\pi d\theta \int_0^{2\pi} d\varphi \int_0^{D_0} dD \int_0^{2\pi} d\Theta N(v_1, \theta, \varphi) V D \Delta u_{||}, \quad (90)$$

when V denotes the relative velocity between the two stars, D the impact parameter, and where, further, the various integrations are, with respect to the different parameters, defining the single encounters. We shall not go into the details here of the evaluation of the multiple integral (90),¹⁰ but only state that on carrying out the various integrations the remarkable result emerges that *to a sufficient accuracy only stars with velocities less than the one under consideration contribute to $\Sigma \Delta u_{||}$* . This result conclusively establishes the fallacy in the assertions made in (a) and, moreover, accounts for the appearance of dynamical friction on our present analysis. Omitting then the details of the analysis we find that

$$\begin{aligned} \Sigma \Delta u_{||} = -4\pi N m_1 (m_1 + m) \frac{G^2}{|\mathbf{u}|^2} & \left(\log \left[\frac{D_0 |\mathbf{u}|^2}{G(m_1 + m)} \right] \right) \\ & \times [\Phi(j|\mathbf{u}|) - j|\mathbf{u}|\Phi'(j|\mathbf{u}|)] \Delta t, \end{aligned} \quad (91)$$

where N denotes the number of stars per unit volume, D_0 the average distance between the stars, Φ and Φ' the error function

$$\Phi(x) = \frac{2}{\pi^{1/2}} \int_0^x e^{-x'^2} dx', \quad (92)$$

and its derivative, respectively, and j the parameter which occurs in the assumed Maxwellian distribution of the velocities

$$\frac{j^3}{\pi^{3/2}} e^{-j^2|\mathbf{u}|^2} d\mathbf{u}; \quad j = \left(\frac{3}{2|\mathbf{u}|^2} \right)^{1/2}. \quad (93)$$

Remembering that $\Sigma \Delta u_{\perp} = 0$ we can write

$$\Sigma \Delta \mathbf{u} = -\eta \mathbf{u} \Delta t \quad (94)$$

where the coefficient of dynamical friction η has now the value

$$\begin{aligned} \eta = 4\pi N m_1 (m_1 + m) \frac{G^2}{|\mathbf{u}|^3} & \left(\log \left[\frac{D_0 |\mathbf{u}|^2}{G(m_1 + m)} \right] \right) \\ & \times [\Phi(j|\mathbf{u}|) - j|\mathbf{u}|\Phi'(j|\mathbf{u}|)]. \end{aligned} \quad (95)$$

In order next to verify directly the existence of a relation of the form (85) we evaluate the sum

$$\Sigma \Delta u_{||}^2. \quad (96)$$

We find that¹¹

¹⁰ The details have since been published in *Astrophysical Jour.* **97**: 255. 1943.

¹¹ "Stellar Dynamics." Equations (2.356) and (5.724).

$$\Sigma \Delta u_{||}^2 = \frac{8}{3} \pi N m_1^2 \frac{G^2}{|\mathbf{u}|^3} \left(\log_e \left[\frac{D_0 |\bar{\mathbf{u}}|^2}{G(m_1 + \bar{m})} \right] \right) \times [\Phi(j|\mathbf{u}|) - j|\mathbf{u}|\Phi'(j|\mathbf{u}|)] |\bar{\mathbf{u}}|^2 \Delta t. \quad (97)$$

Hence,

$$\frac{\Sigma \Delta u_{||}^2}{\eta \Delta t} = \frac{2}{3} \frac{m_1}{m + m_1} |\mathbf{u}|^2, \quad (98)$$

which is to be compared with equation (85). It is thus seen that a detailed analysis of the fluctuating field of the nearby stars in terms of individual stellar encounters idealized, as two body problems, fully confirms the conclusions reached earlier on the basis of certain general principles. In addition we now have an explicit evaluation of the coefficients q and η .

The Principles of Statistical Dynamics

In the two preceding sections we have seen how we can take into account the effect of the near neighbors on the motion of a star statistically through the two coefficients q and η . In thus representing the effect of the near neighbors in terms of the diffusion coefficient q (in the velocity space) and the frictional coefficient η we have abandoned all attempts to describe in detail the motion of any single star and have agreed instead to follow its motion through the distribution function $W(\mathbf{u}, t)$ governing the probability of occurrence of the velocity \mathbf{u} at time t . And as we have already shown, this probability function $W(\mathbf{u}, t)$ satisfies the equation

$$\frac{\partial W}{\partial t} = \text{div}_{\mathbf{u}} (q \text{ grad}_{\mathbf{u}} W + \eta W \mathbf{u}), \quad (99)$$

where it may be recalled that q and η are related according to equation (85). This differential equation satisfied by W leads to an important interpretation of the stochastic process which takes place in the velocity space. For, according to equation (99) we can visualize the motion of the representative points in the velocity space as a *process of diffusion* in which the rate of flow across an element of surface $d\sigma$ is given by

$$- (q \text{ grad}_{\mathbf{u}} W + \eta W \mathbf{u}) \cdot \mathbf{1}_{d\sigma} d\sigma, \quad (100)$$

where $\mathbf{1}_{d\sigma}$ is a unit vector normal to the element of surface considered. We shall find that this interpretation of the stochastic process which takes place in the velocity space as a diffusion process has important consequences for the applications of the theory (see Part III).

So far, we have restricted ourselves to what happens in the velocity space. We have, moreover, assumed that no external forces were acting. The question now arises as to how we can incorporate in a rational sys-

tem of dynamics the stochastic variations in the velocity which a star suffers on account of the fluctuating force acting on it. It is evident that to build such a system of dynamics what we need is essentially a differential equation which will be appropriate for discussing the probability distribution in the six dimensional phase space in contrast to equation (99) which operates only in the velocity space. In other words, we require a proper generalization of Liouville's equation of classical dynamics to include terms corresponding to the stochastic variations in \mathbf{u} . Such a generalization can be readily found.

Quite generally we may write

$$\left. \begin{aligned} \Delta \mathbf{u} &= \mathbf{K} \Delta t + \delta \mathbf{u}(\Delta t) - \eta \mathbf{u} \Delta t \\ \Delta \mathbf{r} &= \mathbf{u} \Delta t \end{aligned} \right\} \quad (101)$$

where \mathbf{K} denotes the external force per unit mass acting on the star and $\Delta \mathbf{u}$ and $\Delta \mathbf{r}$ the increments in the velocity and position experienced by the star in a time Δt . The interval which is chosen must again be such that it is long compared to the periods of the elementary fluctuations but short compared to the intervals during which \mathbf{u} and \mathbf{r} may be expected to change appreciably. Then analogous to the integral equation (81) we now have

$$W(\mathbf{r}, \mathbf{u}, t + \Delta t) = \int \int_{-\infty}^{+\infty} W(\mathbf{r} - \Delta \mathbf{r}, \mathbf{u} - \Delta \mathbf{u}, t) \Psi(\mathbf{r} - \Delta \mathbf{r}, \mathbf{u} - \Delta \mathbf{u}; \Delta \mathbf{r}, \Delta \mathbf{u}) d(\Delta \mathbf{r}) d(\Delta \mathbf{u}), \quad (102)$$

where $\Psi(\mathbf{r}, \mathbf{u}; \Delta \mathbf{r}, \Delta \mathbf{u})$ denotes the transition probability in the phase space. According to equations (82) and (101) we now have

$$\Psi(\mathbf{r}, \mathbf{u}; \Delta \mathbf{r}, \Delta \mathbf{u}) = \frac{1}{(4\pi q \Delta t)^{3/2}} e^{-|\Delta \mathbf{u} - \mathbf{K} \Delta t - q \mathbf{grad}_{\mathbf{u}} \Delta t + \eta \mathbf{u} \Delta t| - 1/q \Delta t} \times \delta(\Delta x - u_x \Delta t) \delta(\Delta y - u_y \Delta t) \delta(\Delta z - u_z \Delta t). \quad (103)$$

Expanding the various terms in equation (102) in the form of Taylor series and proceeding as in the derivation of equation (83) we obtain

$$\frac{\partial W}{\partial t} + \mathbf{u} \cdot \mathbf{grad}_{\mathbf{r}} W + \mathbf{K} \cdot \mathbf{grad}_{\mathbf{u}} W = \text{div}_{\mathbf{u}} (q \mathbf{grad}_{\mathbf{u}} W) + \eta W \mathbf{u}. \quad (104)$$

The foregoing equation represents the complete generalization of Liouville's theorem of classical dynamics for a single particle. On the left-hand side we have the usual Stokes' operator D/Dt operating on W while on the right-hand side we have the terms incorporating the fluctuations caused by the neighboring stars. It should, however, be noticed that the Liouville equation now operates in the six dimensional phase space. This is because we have taken into account the effect of the neigh-

boring stars statistically through the terms involving q and η . Further, it should be noticed, too, that the relation (85) between q and η ensures that the Maxwell-Boltzmann distribution in the phase space is invariant to the stochastic process considered (see the section below).

Analytical Dynamics versus Statistical Dynamics

In the preceding sections we have outlined the general principles of a statistical theory of stellar dynamics. In order that we may emphasize and further amplify the basic ideas which are involved, we shall contrast the outlook of statistical dynamics with the point of view familiar in analytical dynamics.

ANALYTICAL DYNAMICS

1. In analytical dynamics we follow in *detail* the motion of each of the degrees of freedom of the dynamical system.
2. The notion of acceleration is fundamental to analytical dynamics.

STATISTICAL DYNAMICS

1. In statistical dynamics we follow instead the motion of each of the particles *statistically* when under the fluctuating influence of a large number of other particles belonging also to the system.
2. For the success of the methods of statistical dynamics it is essential that time intervals Δt exist with the property that they are long compared to the periods of the elementary fluctuations but which are at the same time short compared to the times necessary for u to change appreciably. Moreover, during such an interval Δt the mean square increment in u is given by

$$\overline{[\Delta u]^2} = 2q\Delta t.$$

Accordingly

$$\sqrt{\frac{\overline{[\Delta u]^2}}{\Delta t}} \rightarrow \infty \quad \text{as} \quad \Delta t \rightarrow 0.$$

In other words, we cannot properly define acceleration within the framework of statistical dynamics.

3. The equations of motion governing a conservative dynamical system can be thrown into the canonical forms

$$\dot{p}_r = - \frac{\partial H}{\partial q_r}; \quad \dot{q}_r = \frac{\partial H}{\partial p_r},$$

$$(r = 1, \dots, N),$$

where H is the Hamiltonian function. These equations can be interpreted by the statement that the development of a conservative dynamical system represents "the gradual unfolding of a contact transformation" (Whittaker).

3. In statistical dynamics the fundamental assumption is generally made that the stochastic process which takes place can be described as a *Markoff chain*. More explicitly, we suppose that the probability distribution

$$W(r, u, t + \Delta t)$$

at the time $t + \Delta t$ can be derived from the distribution $(W(r, u, t))$ at the slightly earlier time t through an integral equation of the form

$$W(r, u, t + \Delta t) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} W(r - \Delta r, u - \Delta u, t) \times \Psi(r - \Delta r, u - \Delta u; \Delta r, \Delta u) \times d(\Delta r) d(\Delta u)$$

where $\Psi(r, u; \Delta r, \Delta u)$ denotes the transition probability. (The foregoing integral relation connecting $W(r, u, t + \Delta t)$ and $W(r, u, t)$ can be regarded as defining a Markoff chain.) Analogous to the interpretation of the canonical equations in analytical dynamics we may describe a Markoff process as "the gradual unfolding of a transition probability."

4. In the $2N$ dimensional phase space the hydrodynamical flow which can be set up by following each point in this space according to the canonical equations is described by Liouville's theorem. According to this theorem, an initially assigned density

$$W(q_1, \dots, q_N, p_1, \dots, p_N)$$

in the phase space varies according to the equation

$$\frac{\partial W}{\partial t} + \sum_{r=1}^N \left(\frac{\partial H}{\partial p_r} \frac{\partial W}{\partial q_r} - \frac{\partial H}{\partial q_r} \frac{\partial W}{\partial p_r} \right) = 0.$$

4. The probability distribution in the 6-dimensional phase space (i.e., the phase space of a *single* particle) is governed by the equation

$$\frac{\partial W}{\partial t} + u \cdot \text{grad}_r W + K \cdot \text{grad}_u W = \text{div}_u (q \text{ grad}_u W + \eta W u),$$

where K denotes the external force per unit mass acting on the particle, and q and η the diffusion and the frictional coefficients describing the stochastic process which takes place in the velocity space (see §5 below).

5. The order of the system of equations governing the development of a dynamical system equals twice the number of degrees of freedom of the system.
5. In statistical dynamics *almost all* the coordinates are ignored. This ignorance of the coordinates of all the neighboring particles becomes possible only because we are able to represent their influence on the statistical motion of any single particle through the two coefficients q and η . More particularly, the stochastic variations which take place in the velocity space can be described as a general process of diffusion in which the rate of flow across an element of surface $d\sigma$ is given by

$$-(q \operatorname{grad}_u W + \eta W u - KW) \cdot \mathbf{1}_{d\sigma} d\sigma,$$
 where $\mathbf{1}_{d\sigma}$ is a unit vector normal to the element of surface considered.
6. The equations of motion of a conservative dynamical system possess the energy integral

$$H = \text{Constant}.$$
6. The generalized Liouville equation in the 6-dimensional phase space governing the probability distribution $W(\mathbf{r}, \mathbf{u}, t)$ is satisfied identically by the Maxwell-Boltzmann distribution

$$W = \text{Constant } e^{-3(|\mathbf{u}|^2 + 2\nabla)/2|\mathbf{u}|^2}$$
 where

$$K = -\operatorname{grad} \nabla.$$
 It is this circumstance which enables the restoration of a Maxwell-Boltzmann distribution from any arbitrary initial state.
7. When dealing with conservative dynamical systems, dissipative forces are foreign to the notions of analytical dynamics. However, dissipative forces may appear in the discussion of dynamical systems in their *non-natural* forms, i.e., when the system is considered in a reduced number of coordinates after the process of the ignorance of coordinates (Whittaker, *Analytical Dynamics*, p. 57).
7. The occurrence of dissipative forces like dynamical friction in the stochastic variations in the velocity experienced by a particle is essential for the success of statistical dynamics. For, it is precisely on account of the occurrence of the term involving dynamical friction that the restoration and maintenance, for example, of a Maxwellian distribution of the velocities among the particles is made possible. Alternatively, we may express the same thing by saying that the operation of a dissipative force like dynamical friction is exactly what is needed to conserve the energy of the assembly as a whole. This may sound paradoxical at first sight, but it is intimately connected with the fact that in statistical dynamics we have essentially performed an ignorance of the coordinates of the neighboring particles.

III. THE RATE OF ESCAPE OF STARS FROM CLUSTERS AND THE EVIDENCE FOR THE OPERATION OF DYNAMICAL FRICTION

The General Theory of the Rate of Escape of Stars from Clusters

(One of the most important factors in the evolution of the galactic and the globular clusters is their gradual impoverishment due to the escape of stars.¹² Essentially, the mechanism underlying this escape of stars is as follows:

On account of the fluctuating gravitational field acting on a star we should expect that there exists a finite probability for a star to acquire a velocity sufficient to escape from the cluster during any specified length of time t . And if a star should acquire the necessary velocity it would naturally escape from the cluster. We shall now show how, on the basis of the statistical theory developed in Part II, we can evaluate this factor in the evolution of clusters quite rigorously.

To be specific, we shall suppose that in order that a star may escape from a cluster it is only necessary that it acquire a velocity greater than or equal to a certain critical velocity which we shall denote by v_∞ . On this assumption the probability that a star will have acquired the necessary velocity for escape during a certain time t can be evaluated quite simply from the probability $p(v_0, t)\Delta t$ that a star having initially a velocity $|u| = v_0$ at time $t = 0$ will acquire for the *first time* the velocity $|u| = v_\infty$ during t and $t + dt$. For, on integrating $p(v_0, t)$ over t from 0 to t we shall obtain the total probability $Q(v_0, t)$ that the star will have acquired the velocity v_∞ during the entire interval from 0 to t . And finally averaging $Q(v_0, t)$ over the relevant range of the initial velocities, we shall obtain the *expectation* $Q(t)$ that a star will have acquired the velocity v_∞ during the time t .

The advantage of formulating the problem in the manner described above is that the function $p(v_0, t)$ can be determined in terms of a solution of the equation (see equation [99])

$$\frac{\partial W}{\partial t} = \text{div}_u (q \text{ grad}_u W + \eta W u) \quad (105)$$

which satisfies certain appropriate boundary conditions. For, remembering that the stochastic process described by the foregoing equation has a simple interpretation in terms of general type of diffusion process, it is evident that $p(v_0, t)$ will be given by

¹² This fact was first clearly recognized by Ambarzumian and Spitzer. For an account of these earlier discussions see "Stellar Dynamics," §§ 5.3 and 5.4, pp. 250-215.

$$p(v_0, t) = - \left(4\pi q |\mathbf{u}|^2 \frac{\partial W(|\mathbf{u}|, t)}{\partial |\mathbf{u}|} \right)_{|\mathbf{u}|=v_0}, \quad (106)$$

where $W(|\mathbf{u}|, t)$ denotes a spherically symmetric solution of equation (105) which satisfied the boundary conditions

$$W(|\mathbf{u}|, t) = 0 \text{ for } |\mathbf{u}| = v_\infty \text{ for all } t > 0, \quad (107)$$

and

$$W(|\mathbf{u}|, t) \rightarrow \frac{1}{4\pi v_0^2} \delta(|\mathbf{u}| - v_0) \text{ as } t \rightarrow 0, \quad (108)$$

where δ stands for the δ -function of Dirac. We shall now show how we can obtain such a solution.

For the case under discussion we have (see equation [95])

$$\eta = 8\pi N m^2 G^2 \left(\log_e \left[\frac{D_0 |\overline{\mathbf{u}}|^2}{2Gm} \right] \right) \frac{1}{|\mathbf{u}|^3} [\Phi(j|\mathbf{u}|) - j|\mathbf{u}| \Phi'(j|\mathbf{u}|)]. \quad (109)$$

This formula for η can be written more conveniently as

$$\eta = \eta_0 \nu(j|\mathbf{u}|) \quad (110)$$

where

$$\eta_0 = 8\pi N m^2 G^2 \left(\log_e \left[\frac{D_0 |\overline{\mathbf{u}}|^2}{2Gm} \right] \right) \left(\frac{3}{2|\overline{\mathbf{u}}|^2} \right)^{3/2} \frac{4}{3\pi^{1/2}} \quad (111)$$

and

$$\nu(\rho) = \frac{3\pi^{1/2}}{4} \rho^{-3} [\Phi(\rho) - \rho \Phi'(\rho)]; \quad (112)$$

with $\nu(\rho)$ defined in this manner

$$\left. \begin{aligned} \nu(\rho) &\rightarrow 1 \quad \text{as } \rho \rightarrow 0, \\ \nu(\rho) &\rightarrow \frac{3\pi^{1/2}}{4} \rho^{-3} \quad \text{as } \rho \rightarrow \infty. \end{aligned} \right\} \quad (113)$$

Again, since q and η are generally related according to equation (85), we have

$$q = \frac{1}{3} |\overline{\mathbf{u}}|^2 \eta_0 \nu(j|\mathbf{u}|). \quad (114)$$

Returning to equation (105) we introduce the following change of the independent variables:

$$= \eta_0 t; \quad \mathbf{u} = \left(\frac{2}{3} |\overline{\mathbf{u}}|^2 \right)^{1/2} \boldsymbol{\varrho}. \quad (115)$$

Equation (105) now takes the dimensionless form

$$\frac{\partial W}{\partial \tau} = \text{div}_{\boldsymbol{\varrho}} \left[\frac{1}{2} \nu(|\boldsymbol{\varrho}|) \text{grad}_{\boldsymbol{\varrho}} W + \nu(|\boldsymbol{\varrho}|) W \boldsymbol{\varrho} \right]. \quad (116)$$

For a spherically symmetric solution $W(|\varrho|, \tau)$ equation (116) reduces to

$$\rho \frac{\partial w}{\partial \tau} = \frac{\partial}{\partial \rho} \left[\nu(\rho) \left\{ \frac{1}{2\rho} \frac{\partial w}{\partial \rho} + \left(\rho^2 - \frac{1}{2} \right) w \right\} \right] \quad (117)$$

where we have written

$$\rho = |\varrho|; \quad w = W\rho. \quad (118)$$

According to equations (107) and (108) we require a solution of equation (117) which satisfies the boundary conditions

$$w(\rho, \tau) = 0 \text{ for both } \rho = 0 \text{ and } \rho = \rho_m \text{ for all } \tau > 0, \quad (119)$$

and

$$w(\rho, \tau) \rightarrow \frac{1}{4\pi\rho_0} \delta(\rho - \rho_0) \text{ as } \tau \rightarrow 0. \quad (120)$$

Now equation (117) is separable in the variables ρ and τ . Accordingly we write

$$w = e^{-\lambda\tau} \phi(\rho) \quad (121)$$

where λ is for the present an unspecified constant; we then obtain for ϕ the differential equation

$$\frac{d}{d\rho} \left[\nu(\rho) \left\{ \frac{1}{2\rho} \frac{d\phi}{d\rho} + \left(\rho^2 - \frac{1}{2} \right) \phi \right\} \right] + \lambda\rho\phi = 0 \quad (122)$$

If we now let

$$\phi = e^{-\rho^2/2} \psi \quad (123)$$

we obtain

$$\frac{d^2\psi}{d\rho^2} + \frac{d \log \nu}{d\rho} \frac{d\psi}{d\rho} + \left[2 \frac{\lambda}{\nu(\rho)} + 3 - \rho^2 - \frac{d \log \nu}{d\rho} \left(\frac{1}{\rho} - \rho \right) \right] \psi = 0. \quad (124)$$

It is now seen that in order that a solution of the foregoing equation may vanish at $\rho = 0$ and at $\rho = \rho_m$, it is necessary that λ take one of an infinite enumerable set of discrete values

$$\lambda_1, \lambda_2, \dots, \lambda_n, \dots \quad (125)$$

which we may properly call the "characteristic values" of the problem. Further if

$$\psi_1, \psi_2, \dots, \psi_n, \dots \quad (126)$$

denote the solutions of equation (124) which satisfy the boundary conditions (119) at $\rho = 0$ and $\rho = \rho_m$ and belong, respectively, to the values $\lambda_1, \lambda_2, \dots, \lambda_n, \dots$ then it can be readily verified that these solutions form a complete set of orthogonal functions. Without loss of generality we can therefore suppose that these functions are all properly normalized. Consequently, in terms of the fundamental solutions

$$w_n = e^{-\lambda_n\tau} e^{-\rho^2/2} \psi_n(\rho) \quad (127)$$

which satisfy the boundary conditions (119) we can construct solutions

which will satisfy any further arbitrary boundary condition for $\tau = 0$. Thus, remembering that a δ -function can always be constructed in terms of any complete set of orthogonal functions according to

$$\delta(\rho - \rho_0) = \sum_{n=1}^{\infty} \psi_n(\rho) \psi_n(\rho_0), \quad (128)$$

it is evident that

$$u = \frac{1}{4\pi\rho_0} e^{-(\rho^2 - \rho_0^2)/2} \sum_{n=1}^{\infty} e^{-\lambda_n \tau} \psi_n(\rho) \psi_n(\rho_0) \quad (129)$$

satisfies all the boundary conditions of our problem. Corresponding to the solution (129) for w we have

$$W = \frac{1}{4\pi\rho\rho_0} e^{-(\rho^2 - \rho_0^2)/2} \sum_{n=1}^{\infty} e^{-\lambda_n \tau} \psi_n(\rho) \psi_n(\rho_0). \quad (130)$$

Using the foregoing solution for W we can write down the probability function $p(\rho_0, \tau)$. For, since

$$p(\rho_0, \tau) = -2\pi\rho_0^2 \nu(\rho_0) \left(\frac{\partial W}{\partial \rho} \right)_{\rho=\rho_0}, \quad (131)$$

we have

$$p(\rho_0, \tau) = \frac{\rho_0}{2\rho_0} \nu(\rho_0) e^{-(\rho_0^2 - \rho_0^2)/2} \sum_{n=1}^{\infty} e^{-\lambda_n \tau} \left(- \frac{\partial \psi_n}{\partial \rho} \right)_{\rho=\rho_0} \psi_n(\rho_0). \quad (132)$$

To obtain the probability $Q(\rho_0, \tau)$ that a star having an initial velocity corresponding to ρ_0 will have acquired a velocity corresponding to ρ_∞ during the time τ we have simply to integrate equation (132) from 0 to τ . Thus we find that

$$Q(\rho_0, \tau) = \frac{\rho_\infty}{2\rho_0} \nu(\rho_\infty) e^{-(\rho_\infty^2 - \rho_0^2)/2} \sum_{n=1}^{\infty} \frac{1}{\lambda_n} (1 - e^{-\lambda_n \tau}) \left(\frac{d\psi_n}{d\rho} \right)_{\rho=\rho_\infty} \psi_n(\rho_0). \quad (133)$$

Finally to obtain, $Q(\tau)$, that an average star will have acquired the necessary velocity for escape during a time τ , we must average the foregoing expression over all ρ_0 . The final result can therefore be expressed in the form

$$Q(\tau) = \sum_{n=1}^{\infty} Q_n(\tau), \quad (134)$$

where

$$Q_n(\tau) = A_n(1 - e^{-\lambda_n \tau}), \quad (135)$$

and

$$A_n = \frac{1}{2\lambda_n} \rho_\infty \nu(\rho_\infty) e^{-\rho_\infty^2/2} \left(- \frac{d\psi_n}{d\rho} \right)_{\rho=\rho_\infty} \left[\frac{e^{\rho_0^2/2}}{\rho_0} \psi_n(\rho_0) \right]. \quad (136)$$

The Evidence for the Operation of Dynamical Friction

We shall now illustrate with some numerical results the theory developed in the preceding section.

Now, since in a star cluster the root mean square velocity of escape is twice the mean square velocity of the stars in the system,¹³ it is clear that the values of ρ , which come under discussion are in the general neighborhood of

$$\rho_{\infty} = \sqrt{6} \sim 2.45. \quad (137)$$

For values of ρ_{∞} in this neighborhood it was found that $Q(\tau)$ can be represented with ample accuracy by the first term on the right-hand side of equation (134). Accordingly it was sufficient to specify the lowest characteristic value λ_1 (for a given ρ_{∞}) and the normalized characteristic function ψ_1 belonging to it. In this manner it was found that

$$Q(\tau) = 1 - e^{-0.0075\tau} \quad (\rho_{\infty} = 2.4518). \quad (138)$$

(The foregoing equation provides sufficient accuracy for $\tau > 5$).

Since $Q(\tau)$ gives the expectation that an average star will have escaped during a time τ (in units of η_0^{-1}) we can properly regard $(0.0075\eta_0)^{-1}$ as a measure of the half-life of the cluster. Thus

$$\text{Half-life of a cluster} \simeq 133 \eta_0^{-1}. \quad (139)$$

For the Pleiades η_0^{-1} is of the order of 2×10^7 years, so that its half-life is of the order of 3×10^9 years. In judging this value it should be remembered that (as may be readily verified) when dynamical friction is ignored, a half-life for the Pleiades of the order of only 5×10^7 years is predicted. There can thus be hardly any doubt that dynamical friction provides the principal mechanism for the continued existence of the galactic clusters like the Pleiades for times of the order of 3×10^9 years. But, even with dynamical friction properly allowed for (as we have done), it will be hard to account for such clusters half-lives of the order 10^{10} years. This, in turn, provides another strong argument in favor of the now currently adopted "short time scale" of the order of 3×10^9 years.

In concluding this essay, we might draw attention to the far reaching analogy which exists between these newer methods in stellar dynamics and methods long familiar in the theory of Brownian movement. However, while parts of the theory of Brownian motion are heuristic and appeal to intuitive considerations, it appears that in stellar dynamics the entire problem can be analyzed explicitly in all its phases.

¹³ Cf. "Stellar Dynamics," pp. 206-207.

STUDIES ON FRESH-WATER BRYOZOA. XIV
THE OCCURRENCE OF *STOLELLA INDICA*
IN NORTH AMERICA *

By

MARY D. ROGICK†

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* Publication made possible through grants from the income of the Publication Fund and the George Herbert Sherwood Memorial Fund.

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INTRODUCTION

The purpose of the present study is to report the occurrence of *Stolella indica* Annandale 1909 in North America. It was collected in Arboretum Pond close to the Westtown School in Westtown, Chester County, Pennsylvania, on September 10, 1940. It is a sincere pleasure to acknowledge the many and most gracious courtesies extended by Mr. and Mrs. George Lower of Westtown to the author during the collecting trip.

The genus *Stolella*, to date, has been reported from Asia by the late Dr. Nelson Annandale, from South America by Dr. Ernst Marcus. The present finding extends its range to a third continent—North America.

There is some question as to the number of species in this genus. Thus far, three have been described. Annandale reported *Stolella indica* from Bulandshahr in the United Provinces, and from around Calcutta in 1909, and from Madras in 1915. He reported *Stolella himalayana* from Malwa Tal, Kumaon, in the West Himalayas in 1911. Marcus very completely described *Stolella evelinae* from Pirassununga, in the interior of the State of São Paulo, Brazil, in 1941. However, Annandale's description of *Stolella indica* does not seem sufficiently distinct from his *Stolella himalayana* to justify making two species. The *S. himalayana* is more adequately described than *S. indica*, although both descriptions could be more complete. Some of the illustrations accompanying both descriptions could serve equally well for either species. The Westtown specimens of *Stolella* show various intergrading conditions between the two extremes represented by Annandale's two species. It is the writer's opinion, based on observations on the Westtown material (which seems to fit almost equally well into either of the two Annandale species), that *S. indica* and *S. himalayana* are one species. Very likely, someone will settle the question at some future date by a careful examination of the type specimens which Dr. Annandale had deposited in the Indian Museum.

Stolella indica colonies were fairly abundant on submerged twigs in Arboretum Pond at Westtown, Pennsylvania, in early September. The pond is very small, shallow, quiet, and fed by a spring which passes through a meadow, under a road, then comes out again to well up into the pond. The pond has much submerged pond-weed material and, at one end, some lily pads. Filamentous algae, *Spirogyra*, *Melicerta* and *Epistylis* colonies were abundant on submerged twigs, logs and other objects.

OTHER WESTTOWN BRYOZOA

Incidentally, only a short distance away from Arboretum Pond, is a considerably larger body of water—Westtown Pond—which contained four Bryozoan species: *Paludicella articulata*, *Fredericella sultana*, *Hyalinella punctata* and *Pectinatella magnifica*. These four species were collected on September 9, 1940. The *Paludicella* colonies, small in size and not too abundant, grew on stones in a very shallow part of the pond, where a thin layer of pine needles had accumulated. *Pectinatella* colonies were especially abundant elsewhere in the pond, where it was a little deeper and where there were more twigs and branches partly submerged. *Fredericella* was collected from rocks, while *Hyalinella* occurred on submerged twigs. A number of floating statoblasts of *Hyalinella* were saved for subsequent experimentation. Some of these statoblasts were dried and allowed to remain so at room temperature for 289 days. They failed to germinate when placed under conditions suitable for germination. Others were placed in an ice-box for 289 days, 272 days of which they were dry. When placed under conditions suitable for germination they hatched (Rogick, 1941b, p. 374, TABLE 1, *Hyalinella*). Some of these germinated *Hyalinella* individuals were figured (*ibid.*, p. 371, FIGURES 1-5).

Since the four species from Westtown Pond are well-known forms to bryozoologists, there is no need to discuss them further. The remainder of the article will be devoted to the *Stolella indica* from Arboretum Pond.

STOLELLA INDICA ANNANDALE 1909

In 1850, Hancock described *Plumatella allmani*, a species whose zoarium bears a strong resemblance to *Stolella*, but whose free statoblasts are considerably longer in proportion to their width than those of *Stolella indica* (Hancock, 1850, PLATE III, FIGURE 5). Allman (1856, pp. 106-107) does not include a picture of this statoblast but does reproduce the colony figure and description. Anyone seeing the figures of the colony, but not of the statoblast of *P. allmani*, might readily mistake some of the *Stolella* zoaria for Hancock's species.

The following description of *Stolella indica* is based on the Westtown specimens. Comparisons between it and Annandale's two species will be made as the description progresses.

ZOARIUM.—The Westtown *Stolella indica* colonies are coarse and closely adherent, but can be scraped away from the substratum with ease. They form loosely intertwined, openly branched, or tangled

sprawling growths on the substratum, which may be submerged twigs, rocks, bark, etc., as in *Stolella himalayana*. Their color is pale or grayish yellow. Their branching may be curved, as in FIGURES 1 and 6, or more angular, as in FIGURES 3, 5 and parts of 8, or in a straight unbranched line, as in FIGURES 2, 4 and end fragments of some of the others. One colony may sometimes show all three types of branching.

The number of zooecia along a line or branch may vary. Zooecia may occur in singles, one after the other, or in single file, as in FIGURES 2 and 6, or in groups, as in FIGURES 1, 4, 7 and 8, separated from successive groups of slenderized zooecial bases termed "pseudostolons" by Dr. Annandale. In the Westtown specimens, the zooecia occur more commonly in groups of twos rather than as singles. No groups of threes were found in them as yet, although both Dr. Annandale and Dr. Marcus mentioned them as being present in their *Stolellae*. Annandale figured singles and groups of twos for *Stolella indica* (1911, PLATE V, FIGURES 3 and 4) and for *Stolella himalayana* (1911, FIGURE 49A, p. 246 and 1915, PLATE III, FIGURE 1) and threes for each species (1909, p. 279 and 1911, FIGURE 49A, p. 246). Marcus shows singles, groups of twos, threes and fours for *Stolella evelinae* (1941, PLATE XII, FIGURES 52, 53 and 56).

Although Annandale specified that *Stolella indica* had neither lateral nor vertical branches (1911, p. 229), one wonders whether that was because of the small extent of the substratum on which the colonies grew (duck-weed roots or stems of other plants), or because of the smallness of the colonies, or because of other factors. FIGURES 1 and 8 show some Westtown individuals with both vertical and lateral branches, as in *Stolella himalayana*, although the vertical ones are not abundant and become attached when they come in contact with substratum or bryozoan material. Branches may arise either as extensions of the zooecial base or farther up along the zooecium. Where they arise from the erect upper part of the zooecia (FIGURES 1, 3 and 5) they may curve about in front or back of the zooecia before eventually attaching to the substratum. This is in accord with Dr. Annandale's statement regarding *S. himalayana* (1911, p. 247), that "the zooecia and their basal tubules grow over one another and often become strangely contorted."

ZOOECIA.—The zooecia are, as a rule, of considerable length and varying diameter, although sometimes they may be small and short (FIGURE 6). TABLE 1 gives the length, width and other zooecial measurements. The zooecia are narrowest in the most proximal region of the pseudostolon and gradually widen toward the tip, where they become cylindrical or nearly so (FIGURES 14, 17 and 23). Naturally, in living evagi-

TABLE 1
Stololla indica MEASUREMENTS

	Maximum in mm.	Minimum in mm.	Average in mm.	Number of readings
Free statoblasts (Floatoblasts)				
Total length	0.41	0.35	0.376	44
Total width28	.24	.257	41
Capsule length305	.26	.273	42
Capsule width24	.19	.209	42
Float, dorsal side, length10	.08	.087	26
Float, dorsal side, width06	.03	.045	29
Float, ventral side, length08	.05	.063	23
Float, ventral side, width05	.03	.037	24
Thickness of mid-region16	.12	.134	11
Sessile statoblasts (Sessoblasts)				
Total length56	.47	.509	8
Total width36	.22	.309	8
Capsule length455	.38	.423	8
Capsule width28	.20	.251	8
Float diameter05	.03	.041	8
Pseudostolon diameter, at thin- nest point30	.09	.165	30
Zooecial tube diameter, at # — # point, as illustrated in FIGURE 7	.68	.33	.496	26
Zooecial tip thickness at thickest part67	.44	.521	27
Height of erect zooecial part, from base to invaginated tip, as shown in FIGURE 3 at * — *	2.87	.945	1.744	35
Length from origin of one basal pseudostolon to the origin of the next basal one, as the & distance in FIGURE 1	4.55	1.40	2.74	53

nated specimens, the free part of the zooecium would appear more slender than the tip in retracted ones.

The zooecia are rarely straight but are either bent upward at an angle or curved throughout (FIGURES 1, 3, 5 and 6). The proximal half of the zooecium, including most or all of the pseudostolon, is attached to the substratum, while the distal half is directed away from the substratum upward at varying angles, backward, forward, sideward, or

else swung around like a comma. This growth habit is found in all three *Stolella* species described to date.

The attached basal part of the zooecium is flat. The shape of the dorsal part differs. In cross section the shapes of the zooecia range from a slightly flattened circle to a somewhat rounded wedge or triangle. The roughly triangular shape is due to the keel which is present in a number of zooecia but which is usually not very conspicuous. A faintly discernible emarginate orifice is present in some but not in all zooecia. In these respects the Westtown *Stolella* resembles Dr. Annandale's *S. indica* somewhat more than his *S. himalayana*. This emargination, when present, was formed by a slight thinning of the encrusting ectocyst material.

ECTOCYST.—Cross examination of the Westtown *Stolella* reveals two layers of the ectocyst—the outer encrusting gray or hyaline material and the inner yellow-to-amber colored cuticle—in untreated or preserved specimens. If more layers are present, histological techniques would be required to demonstrate them. The ectocyst is generally very flexible. Taken as a total unit it has a somewhat grayish yellow color. The outer encrusting layer consists of tiny but different-sized hyaline or silicious particles or fragments and debris unevenly distributed over the underlying cuticle (FIGURE 9). They are distributed often in lines or bands (FIGURES 14 and 17), with less heavily encrusted lines or grooves running in various directions. These lines or bands may run circularly around the zooecial tip, diagonally downward from the keel region, parallel with the keel, or occasionally in whorls. The very tip of the zooecium is transparent, or nearly so, because it is little encrusted. The encrusting layer may wear off at the keel, near the orifice and occasionally in the pseudostolon septum region, exposing part of the underlying cuticle which is a chitinous layer of slightly varying thickness (FIGURES 9, 15 and 16). The cuticle appears homogeneous in most places, but in some areas, at least, under favorable circumstances, it appears to be longitudinally striated or laminated (FIGURES 15 and 16). Dr. Davenport (1893, PLATE 1, FIGURE 4; PLATE II, FIGURES 8, 16 and p. 4) observed two types of cuticula, both of which appeared laminated at times, in the stalk of *Urnatella gracilis*.

ENDOCYST.—The soft endocyst layer is ordinarily continuous from one zooid to the next, since the interzooecial septa are not complete but are ledgelike internally. Their lumen is lined with the endocyst (FIGURE 16). The endocyst appears to be more closely applied to the ectocyst in the basal and pseudostolon regions than in the tips of retracted zooecia (FIGURE 23).

POLYPIDES.—Since the *Stolella* specimens had to be preserved soon after collection, there was no opportunity to study them in the living state. Because practically all polypides were in the retracted condition, the exact number of tentacles could not be accurately determined, but judging from the very few partly evaginated individuals (FIGURE 7) the number seems closer to that for *Stolella indica* (30–35, Annandale) than for *Stolella evelinae* (44–64, Marcus).

The previous reference to *Stolella indica* tentacles as being "short, stout, and sometimes slightly expanded at the tips" (Annandale 1911, p. 230), sounds very much as if degenerating or starved individuals rather than normal or prime polypides were described. Bryozoa have the tendency to shorten and thicken their tentacles and other parts as degeneration, starvation, or other adverse conditions set in or proceed (Rogick 1938, PLATES I, II, III), so that one may find both long and slender tentacled individuals and shortened, stubby tentacles in colonies collected at the same time.

The polypides are very much like those of *Plumatella* in size, musculature and organs. Those of a group retract into the same body cavity since there is no complete partition between the separate zooecia.

PSEUDOSTOLONS.—The limits of this term are not exactly definable. The pseudostolon includes a narrow, short extension from the parent zooecium (FIGURE 9), the septal region (FIGURES 9, 15 and 16), and a varying stretch of the narrow proximal part of the next zooecium (FIGURES 1–8, 14 and 23). Its length is not fixed. Its diameter at the thinnest point ranges from 0.09 mm. to 0.30 mm. (TABLE I). The pseudostolon is not uniform in diameter throughout its extent (FIGURES 1–8, 14 and 23). In some places, it may be exceedingly narrow, while, in others, it may be almost as broad as the rest of the zooecial tube. Sometimes it may be reasonably straight (FIGURE 4), but usually it is bent (FIGURES 1, 3 and 23) or quite roughened in outline.

One, two or three pseudostolons or branches may arise from either the extension of the zooecial base or from slightly higher up along the free part of the zooecial tube (FIGURES 1, 5, 8 and 14), just as in *S. himalayana* (judging from Annandale's figures and photographs). Their facility for adhesion to each other and to parts with which they come in contact is borne out by the appearance of the colonies. However, this adhesion is not so firm or permanent that the adherent parts cannot be disengaged from each other by dissection or separation with dissecting needles.

Annandale did not observe any septa in his specimens, but septa are present in the Westtown specimens (FIGURES 9, 15 and 16), although

they are exceedingly difficult to see unless the outer encrusting layer is rubbed off or missing. They may differ in color, thickness and extent. Some are pale yellow, others are distinct amber color. The former are thinner and more delicate than the latter. Some form an extremely narrow, circular, crescent-shaped or semicircular ledge, while others (as in FIGURE 16) are more extensive.

FLOATING STATOBLASTS (FLOATOBLASTS).—Fully developed floating statoblasts were fairly abundant in the Westtown *Stolella* colonies at this time of year (FIGURES 9–13). They are of the same general type as those of *Plumatella* and *Hyalinella* and agree with Dr. Annandale's diagrams and descriptions for *S. indica* and *S. himalayana*. Of *S. himalayana*, Dr. Annandale wrote (1911, p. 247): "Only free statoblasts have been observed. They resemble those of *indica*, but are perhaps a little larger and more elongate." Since no statoblast measurements accompanied the original descriptions of either species, and since there appears to be so much similarity between their free statoblasts and their zooecial characters, it seems logical to assume that Dr. Annandale was perhaps dealing with slightly extreme forms of *Stolella indica*, rather than with two separate species. Measurements for *Stolella indica* from Arboretum Pond are given in TABLE 1. A large majority of the statoblasts studied were very close to the average measurements as given therein.

Stolella indica free statoblasts are slightly more convex on the ventral side than on the dorsal. The dorsal annulus encroaches a little more on the capsule than does the ventral one. The dark capsule is marked on both faces as indicated in FIGURE 13. These statoblasts occur in all parts of the colony, in zooecia where there are living polypides, and in tubes where polypides have completely degenerated and disappeared. Dr. Marcus' keen observations on his living *Stolella evelinae* colonies revealed a vestibular pore through which statoblasts and perhaps other products may be expelled.

SESSILE STATOBLASTS (SESSOBLASTS).—These were found in far fewer numbers in the Westtown colonies at this particular time than were the free statoblasts. Both types may occur in the same zooecial tubes (FIGURE 17). The sessile statoblasts are larger than the floating ones as measurements in TABLE 1 show. The capsule is large, dark, and covered with mammillate markings on the free side (FIGURES 18–22). The vestigial annulus also is marked, but differently. It does not have true "air cells," such as are present in the floating statoblasts, but does have somewhat similar markings, slight thickenings of the chitinous lamella. FIGURE 19 shows the most typical shape and proportions for sessile statoblasts.

BRYOZOAN STATOBLASTS IN GENERAL

For a number of years, bryozoologists have struggled with an unwieldy terminology, using the term statoblast preceded by various qualifying adjectives, like free, floating, sessile, fixed, attached, spiny, barbed, etc., repeating the whole cumbersome combination innumerable times. For this reason, it is suggested that a specific name be given each type of statoblast, thus eliminating the excessive repetition of the term statoblast and reducing the number of adjectives necessary to accurately describe the type of statoblast in question. The terms suggested are a contraction of or a slight change in those now in use. They are: floatoblasts, sessoblasts and spinoblasts.

FLOATOBLASTS.—These are free or floating statoblasts which do not possess spines, barbs or hooked processes (FIGURES 10–13). When mature they are annulated; the float generally consists of “air cells”; the capsule is usually noticeably smaller than the entire statoblast, with the possible exception of the thin-walled statoblasts of *Plumatella casmiana* (Rogick, Study X, FIGURES 2, 3 and 4); and the statoblasts are free or borne about in the body cavity of the animal until released to the outside by various methods. The approximate range of the floatoblasts’ length (taking into consideration all species) is roughly from 0.21 mm. (Kraepelin 1887, p. 113, *Plumatella*) to 0.7 mm. (Rioja 1940, p. 587, *Hyalmella punctata*). Genera producing floatoblasts are *Hyalinella*, *Plumatella*, *Stephanella* and *Stolella*.

SESSOBLASTS—These are sessile, fixed, or attached statoblasts. They may be with or without an annulus, have a relatively large capsule as compared with their outside measurements (FIGURES 18–22), and are fastened to the zoecial wall with cementing material, thus remaining permanently attached to the substratum long after the colony has died or has been worn away from its base. The approximate range in length of sessoblasts varies from 0.27 mm. (Study IX, p. 195, *Fredericella sultana*) to 0.64 mm. (Harmer 1913, p. 452, *Plumatella fungosa coraloides*). Genera producing sessoblasts are *Fredericella*, *Hyalinella*, *Plumatella*, *Stephanella* and *Stolella*.

SPINOBLASTS.—These are floating or free statoblasts provided with spines, barbs, or hooked processes. They are much larger than floatoblasts, ranging in size from approximately 0.7 mm. (Kraepelin 1887, p. 153, *Cristatella mucedo*) to 1.75 mm. (Annandale 1910, p. 57, *Pectinatella burmanica*). Genera producing spinoblasts are *Cristatella*, *Lophopodella*, *Lophopus* and *Pectinatella*.

SUMMARY

1. Five Bryozoan species were collected from Westtown, Pennsylvania, on September 9 and 10, 1940. They are:

Paludicella articulata (Ehrenberg 1831)

Pectinatella magnifica Leidy 1851

Fredericella sultana (Blumenbach 1779)

Hyalinella punctata (Hancock 1850)

Stolella indica Annandale 1909

2. The finding of *Stolella* here extends the range of this genus to a third continent, North America, and makes possible additional comment on the relationship between *Stolella indica* and *Stolella himalayana*.

3. *Stolella indica* and *Stolella himalayana* are thought to be the same species.

4. Additional measurements, diagrams and variations for *Stolella indica* are given.

5. A slight change in statoblast terminology is suggested. The terms floatoblast, sessoblast and spinoblast, are introduced to replace the longer and more cumbersome combinations of adjectives and nouns heretofore used.

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EXPLANATION OF PLATES

All figures are of *Stolella indica*, which was collected in Arboretum Pond, West-town, Chester County, Pennsylvania, on September 10, 1940, and were drawn with the aid of a camera lucida.

For FIGURES 1 through 8, 14, 17 and 23, a 5x ocular and a 4x objective were used, but the sketches were reduced to varying sizes before being permanently drawn. Consequently, scales for the various figures will be different. Please refer to "Abbreviations" for information on which scales apply to which figures.

For FIGURE 9, a 5x ocular and a 10x objective were used, then the sketch was reduced. For FIGURES 15 and 16, a 10x ocular and a 21x objective, while for FIGURES 13 and 22, a 10x ocular and a 45x objective were used. A 10x ocular and 10x objective were used for FIGURES 10 through 12, and 18 through 21.

Abbreviations

A—single zooecium

B—single zooecium with three pseudostolons branching from it

C—a group of two zooecia

D—zooecial growth form and group typical of Dr. Annandale's *Stolella indica* (1909, p. 279 and 1911, FIGURE 45, p. 230)

E—area showing zooecial tendency to grow in a curve or to twist about, rather than to grow in a straight line, as in Annandale's FIGURE 3 (PLATE V, 1911)

F—pseudostolon

G—thicker than average pseudostolon

H—pseudostolon arising from upper region of zooecial tube, near tip

I—tentacles

J—polypide

K—keel

L—floating statoblast, dorsal face

M—floating statoblast, ventral face

N—base of zooecium

O—statoblasts

P—interzooecial partition or septum

Q—annulus

R—ectocyst

S—endocyst

T—passageway through septum

U—scale for FIGURES 1 through 8

V—scale for FIGURES 14, 17, 23

W—scale for FIGURE 9

X—scale for FIGURES 10 through 12, 18 through 21

Y—scale for FIGURES 15, 16

Z—scale for FIGURES 13, 22

&—length of attached basal part of a zooecium, as in FIGURE 1

——region for measurement of length of free part of zooecium, as in FIGURE 3

#—#—region for measurement of diameter of zooecial tube in basal region, as in FIGURE 7

PLATE I

FIGURE 1. A typical, average, well-developed or well-nourished colony of *Stolella indica*, containing a number of retracted polypides. This was originally a tangled, largely adherent mass, but is here shown dissected away from the substratum and straightened out as much as possible to show the mode of branching and other zoarial characters. Some parts of this colony resemble Dr. Annandale's descriptions of *Stolella indica* (1909, p. 279 and 1911, PLATE V, FIGURE 3), as well as his *Stolella himalayana* (1911, p. 246, FIGURE 49). The labels or letters are explained under "Abbreviations."

FIGURE 2. A short, empty segment from a Westtown colony, showing a row of single zooecia. This resembles the *Stolella indica* of Annandale (1911, PLATE V, FIGURE 4). The third zooecium is directed proximally at its tip, indicating that the zooecia are quite flexible and may grow in various directions.

FIGURE 3. Part of a statoblast-loaded colony, which shows considerable regularity of branching, similarity of zooecia, and the angles at which zooecia are directed upward. The colony is more angular than that of FIGURE 1. The proximal zooecia are closer together than those at the edges of the colony. The upper right-hand branch might easily be mistaken for that of a *Plumatella*, because it is more uniformly tubular or cylindrical than the rest of the colony.

FIGURE 4. A short strip of an empty colony which shows unusually long and narrow pseudostolons. It resembles the distal branches of *Stolella himalayana*, as shown in Annandale 1911, p. 246, FIGURE 49A and 1915, PLATE III, FIGURE 1.

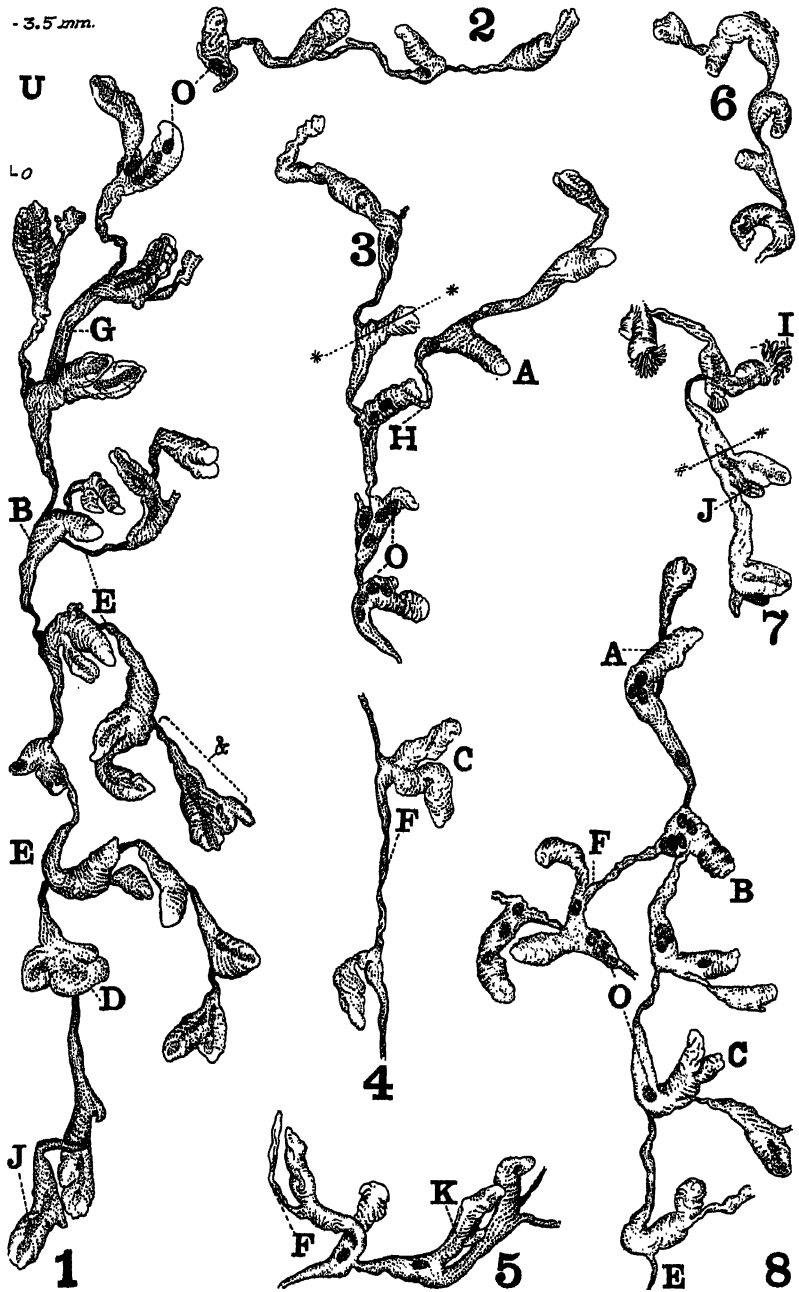
FIGURE 5. Several zooecia showing angular branching but not much swelling of the zooecial tips. The tips are unattached, while the bases were resting on the substratum until they were scraped off. Pseudostolons may cross around in front of or to the side of the parent zooecia, as shown at left. This tendency accounts, in part, for the tangled appearance of some of the colonies. A keel and faint emarginate orifice are shown here, but are not always present in all the zooecia.

FIGURE 6. A small young branch with "curled" single zooecia and extremely short pseudostolons. Compare the zooecial length and the distance between zooecial tips here with the same characters in the other diagrams.

FIGURE 7. A young zoarium with very regular bi-zooecial branching and very swollen tips. The three upper zooecia are partly evaginated, but the exact number of tentacles cannot be easily determined. However, it is greater than the diagram shows. Polypides must be completely evaginated before one can be certain of the number of tentacles.

FIGURE 8. A somewhat more angular colony than that of FIGURE 1. Comparison between it and Dr. Annandale's *Stolella indica* and *Stolella himalayana* shows strong resemblance between them all. All the polypides have degenerated; only free statoblasts and a few degenerate masses are left in the zooecial tubes. The pseudostolons are long. So are the free zooecial tips. Some segments show pairs of zooecia, some singles, linearly arranged. Some zooecial tips are very decidedly inclined forward or proximally. Scale *U* applies to every figure on this plate.

- 3.5 mm.



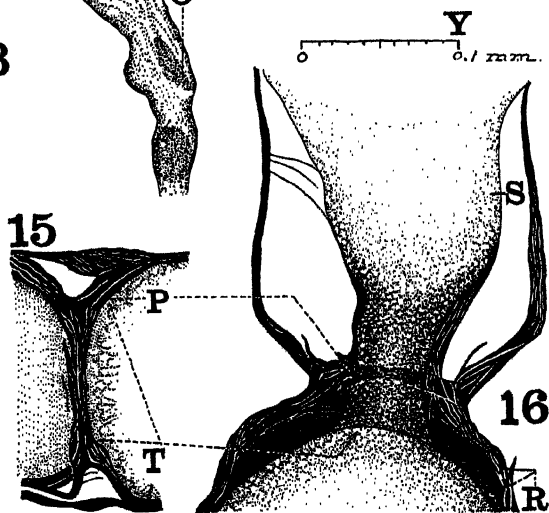
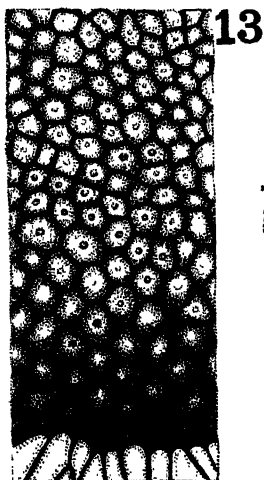
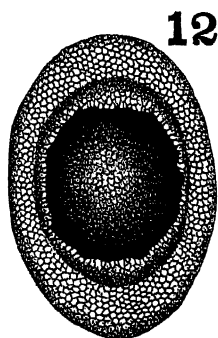
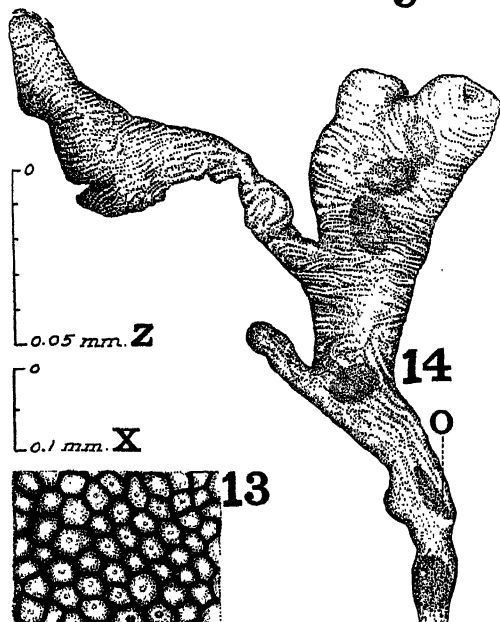
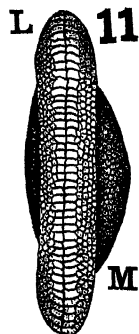
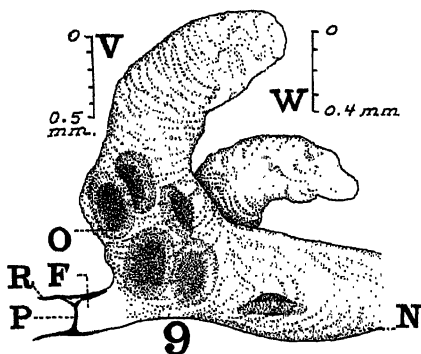
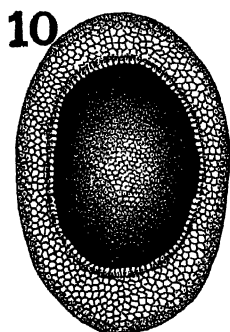


PLATE 2

FIGURE 9. A pair of zooecia whose polypides have degenerated and disappeared. Note the attached basal part (N), the flexible, proximally inclined tips, the six free statoblasts or floatoblasts (O), and the short distal extension of the base which forms a part of the pseudostolon (F). This pseudostolon contains an incomplete septum (P), which is usually scarcely discernible in normally encrusted zooecia, but which is readily seen when the outer encrusted layer of the ectocyst has been worn away to expose the underlying cuticle, as is here the case. A faint keel is indicated by the markings on the ectocyst. Scale W' belongs with this figure.

FIGURE 10. Ventral face of a free statoblast (floatoblast). The encroachment of annulus on capsule is slight. Drawn to Scale X.

FIGURE 11. Edge view of floatoblast. The ventral face (M) is more convex than the dorsal face (L).

FIGURE 12. Dorsal face of free statoblast showing the slightly larger annulus on this side. Drawn to the same scale as the preceding two figures.

FIGURE 13. An enlargement of the floatoblast capsule surface showing the capsule markings. At the bottom is a narrow, light-colored strip belonging to the annulus. Drawn to Scale Z.

FIGURE 14. A somewhat more detailed view of zooecia showing the slightly encrusted ectocyst whose opacity or translucency varies from tip to base and from age. This wrinkled appearance is typical of the ectocyst of most of the individuals. Six floatoblasts show through the wall. Two pseudostolons extend from the zooecial tube at different levels. Drawn to Scale V.

FIGURE 15. A pseudostolon septum, similar to the one illustrated in FIGURE 9, but enlarged. The encrusted layer of the ectocyst is missing. The cuticular ectocyst layer, naturally amber-colored but here shown in black, appears laminated or longitudinally striated in the septum region in some instances. The diameter of the septum lumen through which the endocyst would pass is designated by "T." Scale Y' belongs with this and the succeeding figure.

FIGURE 16. An enlargement of the septum region of another *Stolella indica* specimen showing the endocyst (S) passing through the lumen of the septum. The outer encrusted ectocyst layer has been lost, but the inner chitinous cuticular layer is shown in solid black and in cross-hatching. Here it is especially well-developed, thickened and laminated.

PLATE 3

FIGURE 17. Four statoblasts, three free and one sessile, inside the zooecial tube. The sessoblast is attached to the basal part of the tube. Drawn to Scale V.

FIGURE 18. An enlarged side view of the sessoblast of FIGURE 17. The surface over the capsule is tuberculate. The statoblast base is attached to the zooecial wall with cementing material. This and the following two figures are drawn to Scale X.

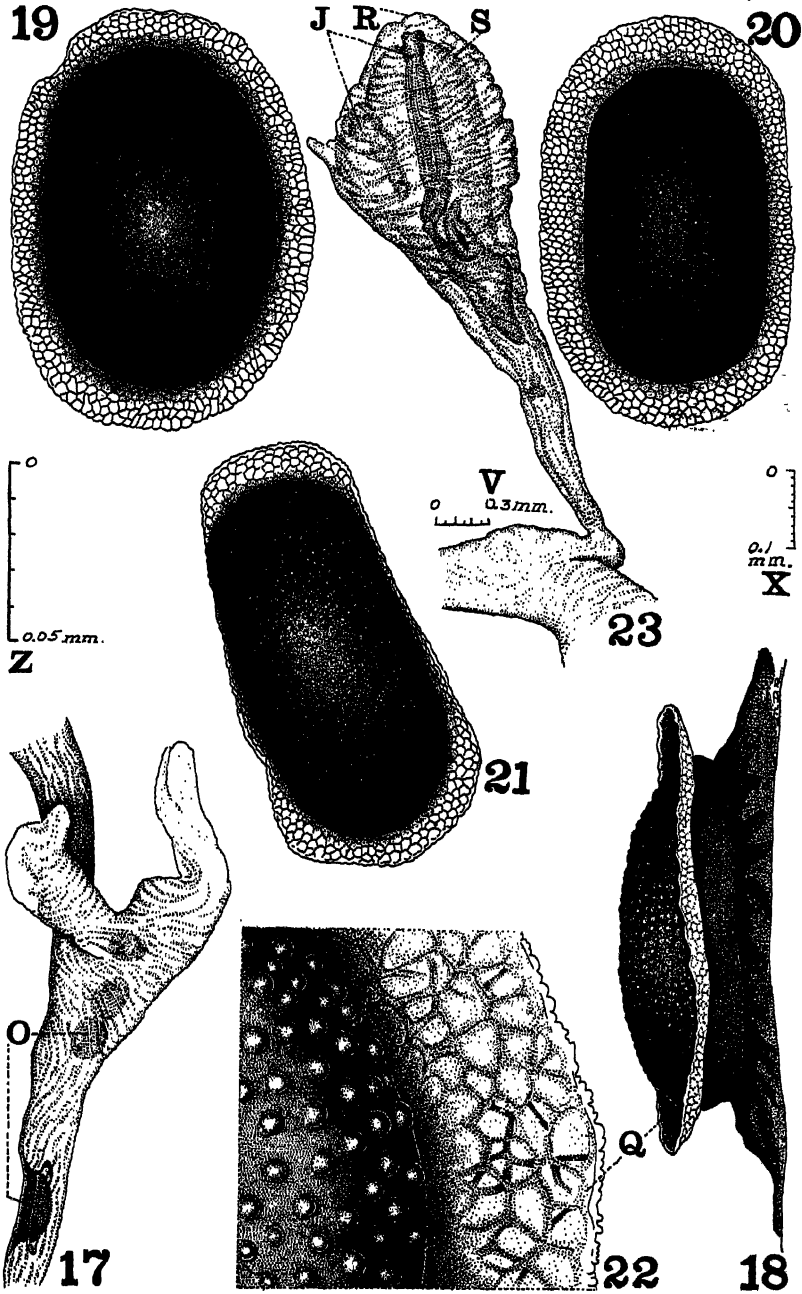
FIGURE 19. The unattached side of a typical sessoblast. The capsule is oval or elliptical.

FIGURE 20. A narrower sessoblast. Two edges of the capsule are more parallel than in the preceding diagram. This appears not to be an uncommon condition.

FIGURE 21. A rather abnormally shaped statoblast found in a narrow part of the pseudostolon.

FIGURE 22. A sector of the annulus and capsule of a sessoblast, greatly enlarged. The annulus has reticulate markings, but not of the same degree of development as those of the floatoblasts. The tubercles on the capsule are very distinct. Drawn to Scale Z.

FIGURE 23. Terminal invaginated zooecia, showing the relationship between ectocyst, endocyst and polypides. The longer polypide is fully developed, the smaller one is immature. The retractor muscles, very much like those of *Phumattella*, have been omitted from this sketch, since they would obscure the digestive tract which is shown here. A small pseudostolon is growing in the direction of the base-line (toward FIGURE 19). Drawn to Scale V.



THE SOCIAL BEHAVIOR OF THE LAUGHING GULL¹

By

G. K. NOBLE and M. WURM²

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¹ This study was supported by a grant in aid of research from the Committee for Research in Problems of Sex, National Research Council.

² Planning and conduction of the study were done jointly by both authors. Interpretation of results and manuscript preparation were carried out exclusively by the junior author. Dr. J. P. Chapin's critical reading of the manuscript is gratefully acknowledged.

Dr. Noble, the senior author, unfortunately died on December 9, 1940.

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INTRODUCTION

The social behavior of gulls has been widely studied. Original work on the herring gull, *Larus argentatus*, by Herrick (1912), Strong (1914) and others has been supplemented by the detailed studies of Portielje (1928), Goethe (1937), Tinbergen (1936, 1937), Darling (1938) and Steinbacher (1938) on the European race of this species. Nevertheless, as recently as 1939, Deusing could still add significant data to our knowledge of the breeding behavior of the herring gull. Kirkman (1937) has devoted a book to the social behavior of the European black-headed gull, *Larus ridibundus*. In striking contrast to the vast amount of work on these species, the social behavior of the laughing gull, *Larus atricilla*, has been very much neglected. While engaged in a study of the relation of hormones to the breeding behavior of this species, we found it desirable to check our laboratory observations against records secured in the field. The present paper represents a summary of the social behavior of the normal bird as observed in the laboratory, supplemented by observations of similar behavior seen in the field.

The laughing gull differs radically from the herring gull in that both sexes of the former species develop, during the breeding season, a black head, reddish bill and eyelids, and dark red legs. Recent studies have shown that the majority of secondary sex characters have some functional significance (Tinbergen, 1939), but no student of gull behavior has suggested a function for the nuptial dress of any black-headed gull. We, therefore, decided to study the laughing gull in two different colonies and to compare its behavior with that of other gulls available to us. Steinbacher (1938) has made some broad comparisons of behavior patterns and calls between different species of gulls maintained in a zoological park. We have studied the courtship of some of the gulls in the New York Zoological Park. Most of our work, however, was done with the laughing gull and only brief comparison will be made with these other species.

ECOLOGICAL PREFERENCES OF THE LAUGHING GULL

We have studied the laughing gull at Muskeget Island, Massachusetts, and at Stone Harbor, New Jersey. The former locality was especially favorable, because more than 20,000 pairs nested there in 1940, as well as approximately 1500 pairs of herring gulls and 3000 pairs of common and roseate terns. Approximately 200 pairs of laughing gulls

attempted to nest at Stone Harbor in 1940, but the conditions were not as good as at Muskeget, high waters restricting the range of the birds and frequently destroying nests. Muskeget is approximately a mile long. The northern third and the eastern fifth are more or less roughened by sand dunes. Dense meadows of beach grass (*Ammophila arundinacea*), mingled with poison ivy (*Rhus toxicodendron*), cover the western and southern portions. The southern and central part of the island is marshy and tends to flood at very high water. Two small fresh-water ponds are found just east of this marshy section. The island was unoccupied in the summer of 1940, but there is a cluster of fishing and hunting shacks in the southeastern section and at the extreme western end of the island.

The herring and laughing gulls displayed distinctly different habitat preferences. The former occupied the dune area and the latter were most abundant in the tangle of beach grass and poison ivy. Correlated with this difference in habitat selection, was the herring gull's habit of alighting often at a distance of 50 yards or more from his *Standplatz* and walking to this lookout and feeding station. The dunes afforded a view over surrounding areas which many observers have emphasized



FIG. 1. Group area and communal courtship ground of laughing gull. On Muskeget Island, nests are radially distributed.

as a requirement of the nesting herring gull. On Muskeget, however, many herring gulls nested on the bare expanses of sand between dunes where there was no driftwood or other material to form a *Standplatz*. On one such sandy stretch, 24 by 10 feet, there were 10 nests but only 5 elevations of any sort which could form an outlook station. Laughing gulls occasionally had an outlook station near the nest, but this was very rare. Many laughing gulls alighted on, or very near, their nests when these were hidden in dense vegetation (FIGURE 1).

Correlated with the preference of the herring gull for comparatively open nesting areas was the fact that this species occupied conspicuously larger nesting territories. The nests of the herring gull were usually 10 to 15 feet apart, although 3 were noted between 6 and 7 feet apart. In contrast, the nests of the laughing gull were usually only 2 to 3 feet apart in the denser vegetation. We counted 5 that were 10, 18, 16, 18 and 12 inches apart in one of the more favored areas.

At Stone Harbor the gullery was dissected by numerous salt-water streams and covered with an even growth of salt-water grass (*Spartina globra*). Here, the distance between nests averaged about 3 feet. In

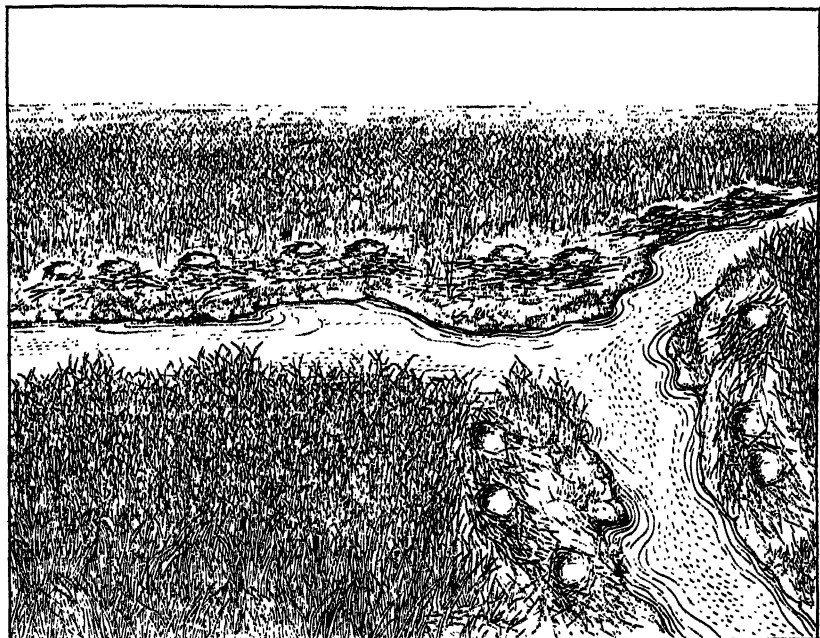


FIG. 2. Group area and communal courtship ground of laughing gull. At Stone Harbor, this area is located on the high ground along the bank, and nests are laterally distributed.

addition to the vegetation, territory size was also restricted by the tides which flood the grounds. No nests or group areas were found in the center of the small islands. Rather, the nests were concentrated along the banks of the streams which offer a slightly higher elevation (FIGURE 2).

COMPETITION BETWEEN THE SPECIES

The herring gull has gradually extended its range toward the center of Muskeget, according to Mr. A. J. Hagar (personal communication). During the last 3 years he has noticed a marked advance. At the west and east ends of the island we noted herring gull nests almost surrounded by nests of laughing gulls. In these cases, there was a slight rise or a ridge of dead vegetation which gave the herring gull a view of the area immediately surrounding the nest. Laughing gulls frequently swoop down at these outposts of the gull colony, but we never observed a herring gull being struck. When we moved several herring gull fledglings into the center of a laughing gull colony, they were severely pecked by the resident birds. A walk across the island revealed little or no fighting between the two species of gull, and because of the decidedly different habitat preferences it is highly improbable that the herring gull will eventually replace the laughing gull. Like the laughing gull, the roseate tern also nests in denser vegetation and has relatively small territorial requirements. Little conflict may therefore be expected between these birds.

The conditions are somewhat different with the terns, which nest on a terrain suitable to the herring gull. During 1940, the largest colonies were near the dwellings at the southeast section of the island. In 1896, the size of these tern colonies was described as follows: . . . "the birds have reached such numbers as to make even an approximate estimate useless" (Mackay, 1897). At this time, the laughing gulls numbered only a few dozen pair. In 1908, Forbush reported the tern population of Muskeget Island to be approximately 10,000, while the laughing gulls then numbered about 1000. Whereas both birds showed an independent increase in numbers through 1920, the prosperity of the terns seemed to have ended when, in 1921, several pairs of herring gulls entered their breeding ground. The herring gull, undoubtedly, is responsible for the death of many tern chicks and destruction of eggs. However, the depressing effect of this species appears to be exerted primarily through a preference for a type of breeding ground resembling that of the common tern, *Sterna hirundo*.

Competition between laughing gulls and herring gulls was at a mini-

num, largely because each occupied a different ecological niche. The behavior of the young also reflected this condition. Correlated with the dusky brown plumage was the fact that laughing gull chicks were confined to the denser vegetation and were rarely observed in the open. Furthermore, they wandered only a short distance from the nest. When they were summoned to feed, the young seldom traveled more than 4 feet toward the calling parent. On the other hand, herring gull chicks were frequently seen in the open. From within the territory, usually the *Standplatz*, the adult summoned its young from distances of about 100 feet. Chicks show little evidence of aggression, although we infrequently observed the young of the laughing gull pecking others, probably in defense of territory. While young gulls sometimes suffered from attacks of terns, many of the deaths were the results of attacks by adult gulls. This is due wholly to the territory defense drive of nesting gulls. Kirkman (1937) has best described this situation in the European black-headed gull. At Stone Harbor, crows visited the laughing gulls in the early morning during the late incubation period, but no direct predations were noted. However, the gulls showed a concerted effort to drive these birds off. A number of adult laughing gulls, with their chests devoured, were also found on Muskeget Island and it is probable that here the short-eared owls, present on the mainland, were responsible.

BEHAVIOR OF THE SEXUALLY INACTIVE LAUGHING GULL

Laughing gulls out of the breeding season were generally arranged in loose flocks and the lack of social activity was in marked contrast to the behavior of breeding birds. Stimuli arising from a food drive and a degree of individual irritability accounted for the few social reactions at this time.

Sudden movement by a bird in the flock was usually met with a chorus of *èh, èh, èh*. This aggressive call was sufficient to prevent severe crowding. Occasionally, the flying momentum of an incoming bird temporarily crowded the group, and, as a result, a number of birds sounded the aggressive note, erected the saddle feathers and attempted to peck. Aggression was also expressed by a slow "long call," *eh-he, he-he-he-he*, produced in a rasping, squeaky voice.

Sexually inactive laughing gulls also used the "food begging" pattern, described below, in which the voice was modified to a shrill *uhe*. This activity was generally confined to competition over food, but was also seen to occur over play objects, such as reeds, twigs, and clam

shells. With the exception of the "choke call" and "moan," the other calls of the breeding bird may infrequently be produced by the sexually quiescent gull in a shrill, squeaky voice.

BEHAVIOR PATTERNS OF SEXUALLY STIMULATED LAUGHING GULLS

In the following description of the behavior of the laughing gull, we have included under each pattern the homologous behavior of other species of gulls as described in the literature. This method is intended to emphasize the interspecies similarities and also to suggest a uniform terminology. We have largely avoided the terms used by earlier observers, for reasons to be indicated below.

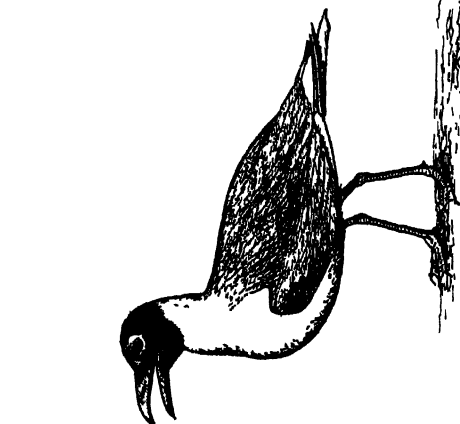
The voices of gulls can be divided into a resonant and a rasping type. The former is represented by the laughing gull (*L. atricilla*), the herring gull (*L. argentatus*), the common gull (*L. canus*), the ring-billed gull (*L. delawarensis*), and the greater black-backed gull (*L. marinus*) in which a large variety of vocalizations is easily recognized. The latter type includes the black-headed gull (*L. ridibundus*) and the Australian silver gull (*L. nova-hollandiae*) in which very few calls can be distinguished.

Our observations of the Australian silver gull have shown that the rasping long call of this species is used in different social situations with only slight modification. Kirkman (1937) likewise found difficulty in designating the calls of the black-headed gull, which he generally indicated as modified "*kuarrs*" or "*kuurps*."

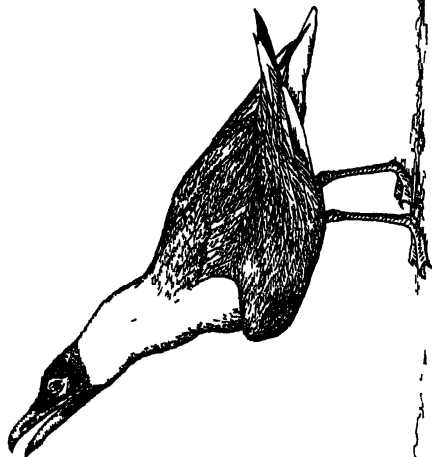
THE LONG CALL.—With the beginning of sexual activity the voice of the laughing gull is the first character to show nuptial modification. The squeaky "slow long call," seldom heard from the sexually quiescent bird, loses its slow, broken or interrupted character, and later becomes loud and clear. This may now be recognized as the true "long call." Its utterance is associated with three sets of vocal and postural elements: (1) the "preliminary long call," *uh-ho, uh-ho*, during which the head and neck are extended in line with the body or at varying angles up to 45° to the ground; (2) the "long call" proper, *how-ow-ow-ow-ho*, during which the head, neck and body form a straight line inclined about 60° to the ground, saddle feathers are smooth and wings are drooped and slightly turned away from the body; (3) the "nasal call," *mnow*, during which the head is simultaneously tossed upward ("head-snap") (FIGURE 3).

The long call of the herring gull has been referred to as the "alarm

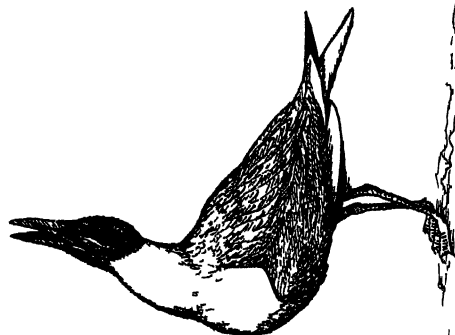
FIGURE 3
LONG-CALL POSTURES



PRELIMINARY
LONG
CALL



LONG
CALL
PROPER



NASAL CALL
AND
HEAD SNAP

cry, jubilation cry" (Steinbacher, 1938), "mating call, love call" (Tinbergen, 1936b), and "courting call, rejoicing call" (Portielje, 1928). Previous writers have recognized that this call consists of three phases, of which the first has been termed by Broekhuysen (1937) the "introduction," while Steinbacher (1938) names it the "enticement call." In *L. atricilla*, the preliminary long call when used independently is termed by us the "flight call." Like the herring gull, a single ring-billed gull at the New York Zoological Park produced the preliminary long call while placing the head between the feet. The long call proper, or second phase, is omitted in *L. delawarensis* and the third phase is produced while pointing the beak skyward and rapidly jerking the head up and down, each time sounding the call *yow*. Such head movements, although not produced in an identical way, are also characteristic of this phase in the call of *L. atricilla* and *L. argentatus*. In the common gull, *L. canus*, Wachs (1933) and Haviland (1914) have independently described a similar call which these observers respectively termed the "passion call" and "vociferous call." Steinbacher (1938) stated that the vocalizations of the herring gull are present in *L. hyperboreus*, *L. fuscus*, *L. dominicanus* and *L. marinus*. For the last-named species Portielje (1928) as well as Darling (1938) described the long call as being louder and deeper than in the herring gull but otherwise comparable. Obviously, the names "alarm, jubilation or passion" are inappropriate since we, as well as Steinbacher, found that the call referred to may be used in aggression, recognition between mates, over food, prolonged incubation or to express general excitement.

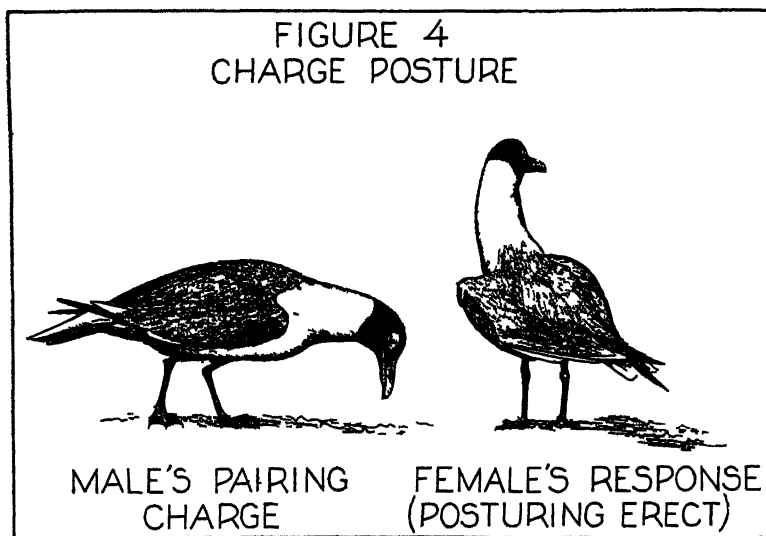
THE FLIGHT CALL.—In addition to loud and frequent long calls, our flock of breeding gulls also showed an increased flying activity. Associated with this we recognized two types of calls: (a) the "flight call," *u-ah, u-ho, u-ahha*, and (b) the "rapid flight call," *bah-ha-ha-ha, bah-bah-bah*.

In the herring gull, the flight call has been variously named by different authors or only phonetically described. Goethe (1937), however, listed a number of vocal reactions to the "migratory call" among which is a "barking" type. This corresponds to the rapid flight call in our bird. The Australian silver gull and the black-headed gull both produce an abbreviated long call which is equivalent to the flight call.

THE WARNING CALL.—This note may be written as *ow! oh! ow!* It is an emphatic loud call, which may be produced by both sexes, but is usually uttered by the male. It is given when birds are reluctant to retreat from the observer or when the nest is approached by a strange bird.

The counterpart for the warning call in the repertoire of the herring gull has been described as a "variant of the enticement call" *kijau* (Steinbacher, 1938), the "fright call and variations" *i-au* (Goethe 1937) and the *au* call of Broekhuysen (1937). The latter author has also identified a warning call in the immature greater black-headed gull as *haee-haee*.

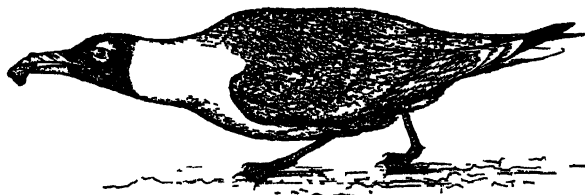
THE CHARGE.—This activity is performed only by the male and can be recognized in two forms: (1) In the "pairing charge" the body is carried in a normal position, the head is slightly lowered and may be extended or kept close to the body and the beak is pointed at the ground (FIGURE 4). In this posture the male "charges," or runs at, and then



stops short of another bird. (2) The "feeding charge" occurs between birds that have formed the initial bonds of a pair (FIGURE 5). For this activity the body is carried low, beak pointed forward, head and neck extended in line with the body or slightly lower. In this attitude the male runs past the female and then "postures" and "flags." Sometimes the feeding charge is performed while the male carries fish and sounds the sex moan, described below.

POSTURING.—From a position in which the head and body are carried normally, the gull suddenly changes to a very erect posture, in which the body is kept horizontal, the neck is stretched vertically to a maximum and the head is kept horizontal, facing away from the bird for

FIGURE 5 CHARGE POSTURE

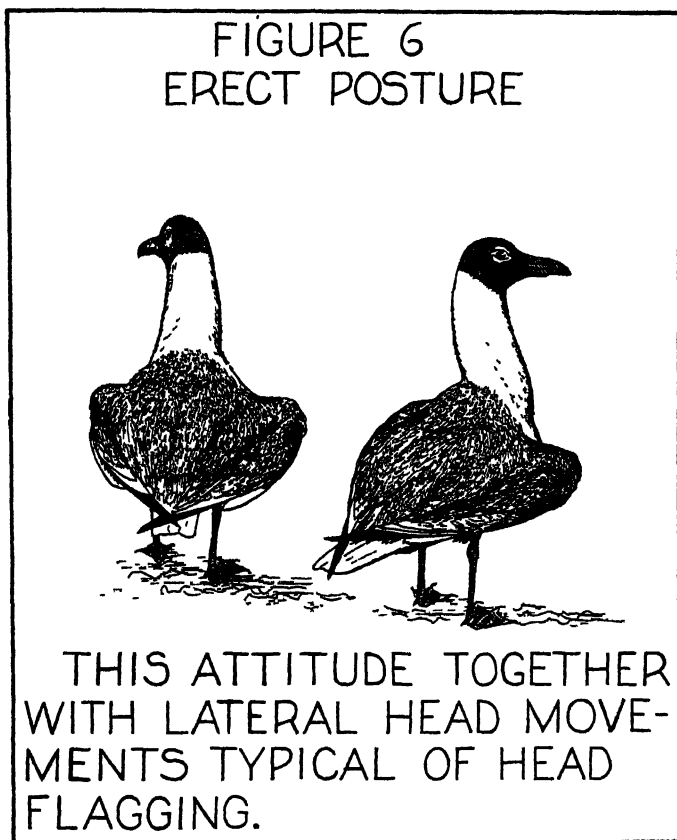


FEEDING CHARGE; MALE CARRIES
FISH AND MOANS.

which the activity is intended. This stance is maintained for a number of seconds during which the wings are drooped, mantle feathers are smooth, and the animal appears rigid and strained. If the bird executes a number of consecutive postures, the head is brought very low before each pose is reassumed.

HEAD FLAGGING.—While the gull maintains an erect posture, the head is slowly and deliberately turned from side to side. The performing bird directs the back of its head toward the bird for which the activity is intended. When two birds simultaneously perform, their bodies form an angle facing away from each other (FIGURE 6). Head flagging, like posturing, is an activity common to both sexes and appears as a formalized ceremony in which the erect posture is accented by the measured lateral movements of the black head.

THE MOAN.—It has been possible for us to distinguish two types of moans, each of which occurs during a distinct social situation. (1) The "aggressive moan," which may be produced by both sexes, though very infrequently by the female, has a short sound which may be written as *uhhhh*, *wuhhhhh*. The aggressive significance of this note can be recognized from the low carriage of head, neck and body, and erection of mantle feathers. It is frequently combined with an active attack. (2) The "sex moan," produced only by the male, has a long plaintive sound with a sharp ending and may be written as *whuooooop!* *whuooooop!* The bird maintains either a semierect or erect posture with



head held close to the neck. Frequently the sex moan is also sounded while the male carries fish during a feeding charge.

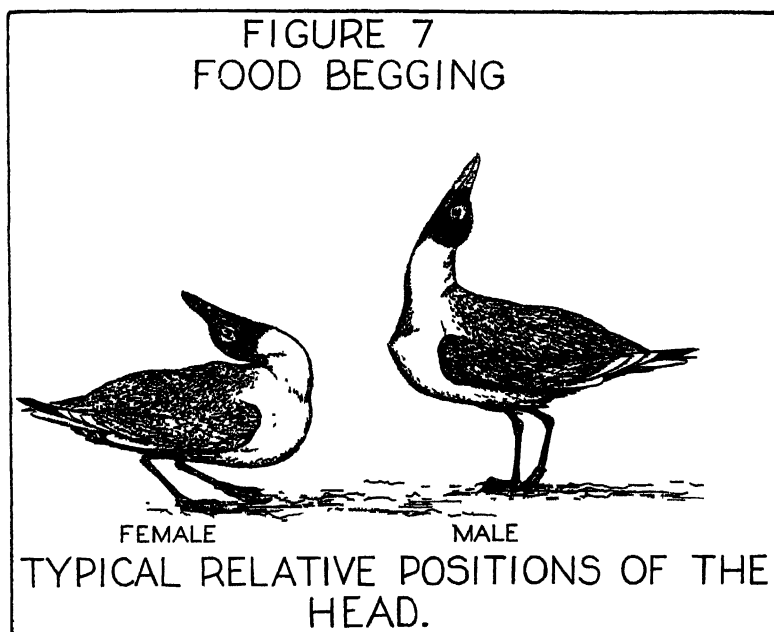
In the herring gull, Steinbacher (1938) described the "nest enticement call" by comparing it to a "cat call," and which he wrote as *maoo*. The nature of the sound and the posture in which it is produced show that this call is equivalent to the sex moan given in the feeding charge posture by the laughing gull. Goethe (1937) referred to the same behavior which he termed the "landing or nesting call" and which he compared with the "whining of an infant." Tinbergen (1936b) also recorded this sound which he compared to a cat call, while Broekhuysen (1937) considered it an expression of tenderness. From a composite account of these authors, we find that the sex moan not only is present in *L. argentatus* but also serves the same function as in *L. atricilla*. It

is used by the male to coax the female before feeding (Goethe, 1937; Portielje, 1928), when returning to the nest to feed female and young (Steinbacher, 1938), and to entice the female to surrender the nest during incubation (Tinbergen, 1936b; Goethe, 1937). In the laughing gull we found the sex moan produced only by the male. Tinbergen and Goethe, however, believed that the female herring gull produced this sound during nest relief and that the male produced it before feeding the female and young. In the greater black-backed gull, Broekhuysen (1937) illustrated the moan (*Zärtlichkeitsausdruck*) in the feeding charge posture but made no mention of sex. Steinbacher (1938) likewise described this vocal and postural behavior in sexually active *L. fuscus*. Haviland (1914) indicated a similar posture and call in the common gull. In *L. ridibundus*, Kirkman (1937) described a *kwoo* note produced in either a food begging or feeding charge posture. As in our birds, this "call note to food" of the European black-headed gull is used by the male to attract the female.

FOOD BEGGING.—The food-begging call resembles the third element in the long call, independently performed. The bird produces the "head snap" and a muted variation of the call which may be written as *nnow*. At the time of intense courtship this note, together with the head snap, may be produced as frequently as 50 times per minute. The female of the pair maintains a posture typical of her sex, in which the body is carried low, tilted forward, and the head is pulled back (FIGURE 7). Food begging is interspersed with gentle attempts at grasping the male's beak and the female nervously runs about her mate as if trying to prevent his escape. During mutual begging the male produces the same call and head snap but is in a semierect posture. Infrequently, we also noted the male initiating food begging. However, the head is always kept relatively higher than that of the female mate.

Food begging has been recognized in *L. argentatus*, and, as in the laughing gull, the call is sounded while the head is tossed (Darling, 1938; Broekhuysen, 1937; Tinbergen, 1936c). We have observed the same behavior in *L. delawarensis* and it is also shown by *L. canus* (Haviland, 1914), *L. marinus* (Broekhuysen, 1937), *L. fuscus* (Darling, 1938) and *L. ridibundus* (Kirkman, 1937).

COPULATION.—After a prolonged period of mutual begging, the male maneuvers toward the tail of the female, mounts and attempts to contact cloacas. Immediately upon mounting, the male engages in vigorous wing flapping and issues the "copulation call," which may be written as *haw-haw-kaw-kaw-kaw-haw*. The female cooperates during copulation by holding the body in a posture resembling that of

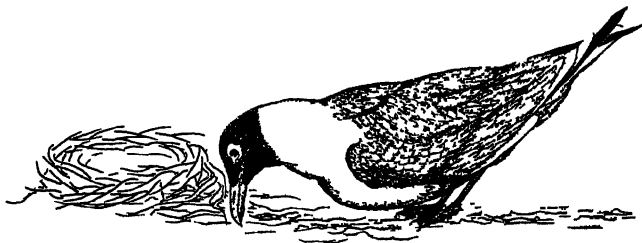


food begging, and sometimes the head is turned in attempts to grasp the male's beak or to peck his breast gently.

In the black-headed gull copulation is accompanied by the usual type of "*kwurps*," but in the herring gull a distinctive "pairing cry" (Steinbacher, 1938) or "copulation rackle" (Goethe, 1937) is produced.

CHOKING.—The choke call and accompanying postures are performed by males and females. During this behavior the body is tilted forward with wings drooped and partly spread (FIGURE 8). The head, with the beak pointing downward, is rapidly jerked, and these movements are accompanied by a call which sounds like *ug, ug, gug, gug, gug*. This pattern is executed by both members of a pair as a mutual ceremony within the nesting area. Frequently, only one bird will produce the choking call, just before squatting on a nest site. When this activity occurs within a pair, the birds maintain an almost sleek plumage. The choking call and posture, however, are also employed between unmated birds. In such instances, antagonism is indicated by a vigorous erection of saddle feathers and wing coverts (FIGURE 9). The activity is initiated by the resident bird in response to an approach

FIGURE 8
CHOKING POSTURE 1

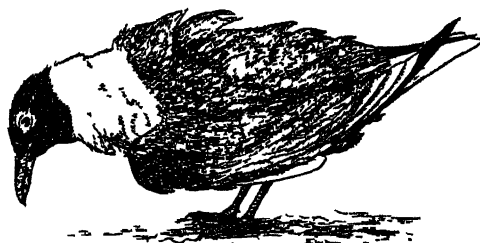


USED IN NESTING AREA; SMOOTH
PLUMAGE INDICATES NON-AGGRESSION.

or attack by a stranger. Sometimes the choking display between unpaired birds is also followed by combat.

In the herring gull choking behavior has been referred to as the "plucking and lying down call" (Goethe, 1937), "crouch and nestling" (Tinbergen, 1936b), "mating call" (Portielje, 1928) and "nestling call" (Steinbacher, 1938). Darling (1938) compared the sound to the cooing of a

FIGURE 9
CHOKING POSTURE 2



WHEN USED IN AGGRESSIVE
DISPLAY, PLUMAGE IS RUFFLED.

pigeon, while Goethe and Steinbacher expressed it as *go-go-yo* or *oog-ogg* and Portielje as *hu-oh*. In all instances, the call is produced while the head is bobbed and may be mutually performed by a pair at the nesting site. Portielje (1928), however, observed this behavior pattern in the absence of a nest and even while birds were swimming. Steinbacher (1938) found that the choke is used by the herring gull at the site of a future nest, at the border of a territory and directly over the nest. In the common gull, *L. canus*, Wachs (1933) observed a "social pecking" associated with nesting. During this activity the birds make downward pecking movements without actually striking the ground. In the lesser black-backed gull (Darling, 1938), the greater black-backed gull (Broekhuysen, 1937) and in the present study of the Australian silver gull the same behavior pattern has been found. In the black-headed gull, Kirkman (1937) also described a *koo-koo* sound produced with jerking movements of the head as if the bird were attempting to disgorge. Again, this behavior was used within the pair and during territory defense by both resident and strange birds.

BEHAVIOR PATTERNS OF SEXUALLY ACTIVE AUSTRALIAN SILVER GULLS

At the beginning of May, 1940, we banded seven captive Australian silver gulls, *L. nova-hollandiae*, maintained at the New York Zoological Park.¹ Since no account of this species has been published, we present the following preliminary study. At the time, these birds appeared to be sexually active and were released in a large outdoor flying cage. Sex could not be accurately determined because an exploratory examination of the gonads was impractical and because no copulations were observed. Attempts at pair formation were frequently observed and several activities can be described. Again, as in the laughing gull, we have, as far as possible, named each activity according to an outstanding character.

LONG CALL.—During the long call, the body and neck are kept in a line pointing upward at about a 60° angle. The head is bent forward from the nape with the beak pointing at the ground and the wings partly extended from the body. The call may be written as *brurrurr-urrurrurr*. Unlike the long call in any other gull studied, this call has only a single element and is produced in a single posture.

¹ In this connection we wish to express our gratitude to Dr. Lee S. Crandall, Curator of Birds at the New York Zoological Park. Dr. Crandall generously provided for our use not only the birds (Australian silver gulls and other species of gulls) but facilities for experimentation at the Park.

FOOD BEGGING.—Although the long call is generally produced as an individual pattern, it is sometimes followed in immediate succession by the food-begging posture. This is identified by the pose in which the body, neck and head are carried very low, and at the same time the neck is arched downward. During this behavior the bird produces a barely audible sound which, however, resembles the long call.

Food begging is demonstrated by both sexes. This activity, like the long call, is used as a sexual display and as an aggressive display. When used in aggression, the head is held relatively high but the characteristic downward arch in the neck is also present. During courtship, one bird retreats and alternates the long call and long-call posture with the food-begging posture. The second bird follows in the rear, slightly to the side, and likewise alternates the long-call posture with the food-begging posture. This is similar to the behavior in the laughing gull, in which the nasal call and head snap (constituent elements of food begging) follow the long call proper without any interruption. When food begging is mutually performed in the laughing gull it may lead to a fight if the proper head positions are not maintained in conformity with a dominant-subordinate relationship. Thus far we have not been able to recognize any finer details in the food-begging behavior of the silver gull.

CHOKING.—In the silver gull, choking behavior resembles that observed in the laughing gull. The body is kept at a normal height parallel to the ground and the head is lowered with the point of the beak resting on the ground. The wings, however, are not extended and, as in every other pattern, no use of feathers has ever been observed. Only on rare occasions is the typical *ug-ug-gug-gug* sound produced. Sometimes the sound resembles a combination of the above and a high-pitched variation of the rasping note of the long call. Usually, the sound consists only of a high-pitched long-call note. While in the choking posture, the head is only rarely bobbed. The movement occurs simultaneously with the *ug-ug-gug* sound and only at times of great stress. Such occasion arises when a strange gull approaches close to a resident bird. Choking behavior is also executed by a bird preparatory to squatting in its territory, when entering its nesting area, after retreating from an attack, and when approached by a courting pair.

As in the laughing gull, choking behavior in *L. nova-hollandiae* is an activity associated with territory. Whether this behavior is also used in nest building could not be ascertained because no nests were constructed during the observational period. However, choking is used as an aggressive display during which it is initiated by the resident bird

and answered with only brief choking by the strange bird. This behavior apparently is also common to both sexes.

FLIGHT CALL.—The flight call, produced by the silver gull in a normal or semierect posture, may be written as *brucrr*, and, as in *L. atricilla*, resembles an abbreviated element of the "long call." In the silver gull, furthermore, the call appears to be exchanged between individual birds which frequently react by cocking the head in a listening position.

PAIRING CHARGE.—The pairing charge is an activity common to both birds of a courting pair but appears to be initiated by the male. With the body in a semierect posture, the male rapidly runs for a short distance toward the intended mate, stops short, and then moves forward in a restrained peck. Sometimes the peck is actually executed. In response to this activity the female assumes a very erect posture, slowly turns around, retreats two steps, lowers the head and postures erect again. The posturing thus functions to inhibit a true attack. It also stimulates similar posturing in the charging bird.

FEEDING CHARGE.—The feeding charge is another pattern of sexual behavior, parts of which can be performed by both sexes. For this, the bird keeps the body, neck and head in a straight line, pointing forward, with the bill almost touching the ground. In this pose, the bird, carrying some fish in its bill, walks toward the intended mate and produces a modified long-call note. When the activity is performed without food in the bill, it frequently is followed by regurgitation. However, only males were observed to carry food or regurgitate.

DEFENSE POSTURE AND FLAGGING.—The assumption of the erect posture was observed as a regular response to the pairing charge. This reaction is not sexually stimulating but serves to prevent the charge from becoming a true attack. The same response may be observed in connection with a normal fight. In spite of the erect posture, a charging male sometimes delivers an actual peck. In such instances the pecked gull retains the erect posture of the body and in addition points the beak straight up. This always inhibits further aggression.

The erect posture is sometimes employed in a more elaborate display which may be sexually stimulating. The gull stands in front of its mate in an erect posture and then turns around two or three times. Because of its significance in the subsequent discussion, the following note is presented:

One bird slowly walks forward in the feeding charge posture with wings spread. About a half minute later another gull approaches, also takes the feeding charge posture and walks directly behind the first bird. Suddenly the rear bird pecks the

wing tip of the bird in front and is answered by an attack. In response to the attack, the rear bird retreats a short distance, postures erect and the front bird now also postures erect. About five seconds later, the attacking bird, still in the erect posture, turns around twice and comes to rest with the tail pointing at the other bird. The feeding charge is again repeated and again a peck is directed at the bird's tail. This is repeated three more times. After each attack, the birds face each other in an erect posture, then the attacking bird turns around 2 or 3 times, stops, facing away from the other bird, and then walks forward in a feeding charge.

The same behavior, preceded by the pairing charge, was observed in other pairs.

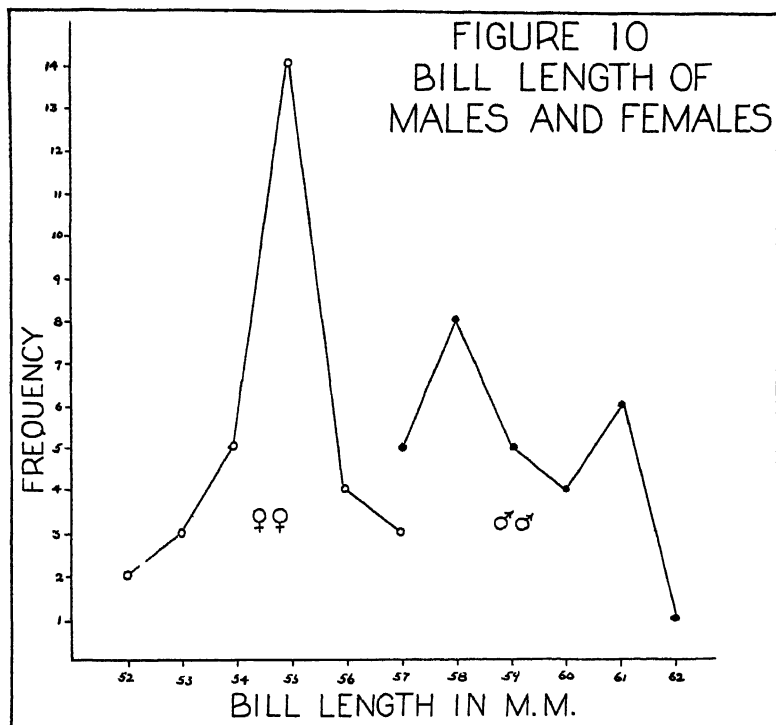
FIELD STUDY OF THE LAUGHING GULL

Our field observations at Stone Harbor, New Jersey, were started on May 18, 1940, and on Muskeget Island, Massachusetts, on June 1, 1940. Two blinds, the shape of truncated pyramids, measuring 42 by 42 by 48 inches, were permanently staked out in regions containing the largest groups of gulls. At Stone Harbor, it was also necessary to use an elevated floor in the blinds because the ground was usually flooded except for a short period at low tide. During the prenesting period it was impossible to capture any birds for marking. Later, it was possible to trap a number of incubating birds for banding, but these could not be recognized in the grass. Other birds, whose beaks were painted and head plumage disturbed, were never seen again. However, we were successful in marking a number of incubating gulls by painting the side of the nest and erecting small reeds dipped in paint.

Identification of Sex

The laughing gull, like other members of the Laridae, is sexually isomorphic. However, we have noted a significant sexual difference in bill size of captive birds. FIGURE 10 represents the frequency of the bill length, measured from the angle of the mandibles to tip of beak when closed. The measurements were based on 17 adult and 12 juvenile males and 20 adult and 11 juvenile females. These showed that the average bill length of males was 59 mm., but most frequently 58 mm., while 55 mm. represented the average and most frequent class for females.

In the field, sex could be identified within groups of two to four birds by relative size and posture. Generally the males stood taller than the females and the size of the body, especially the head, of the male appeared larger than that of the female. Even in larger groups this criterion sometimes facilitated our recognition of some males. On the other hand, the sex differences in these characters frequently were not distinctive. The more positive method of distinguishing the sexes



was based on behavior patterns which included the following: male mounts female during copulation; male keeps head high during mutual food begging and tosses the head a few times, whereas the female shows incessant begging activity during which the head is kept low; only males pass food and only females accept it; only males perform the pairing and feeding charges from which other males always retreat while females may respond.

The sexually specific character of these behavior patterns was first recognized in captive laughing gulls in which sex was determined by direct examination of the gonads. These birds, however, showed no sex differences in body size and posture. This may have been a function of the plumage, since captive birds also failed to show the pink hue observed in the breast of free gulls.

Organization of the Colony

At the start of our field work, we discovered only adult birds in full nuptial plumage. The colony as a whole was divided into a number of

distinct groups which at Stone Harbor averaged about 25 birds. The Muskeget colony showed the same organization and here the largest group was found to contain 98 gulls. The population of these groups, however, was very inconstant. When put to flight, the group returned to occupy its original area within an hour, but the number of individuals in the flock varied from the original. Also, at low tide, the group areas were generally found deserted because there occurred mass excursions to the feeding grounds. At other times, small numbers of birds and even individuals would enter or fly off from the group. It was not possible to ascertain whether a gull was a constant member of one group, although individual birds were painstakingly observed in flight for long periods.

During the early part of the breeding season, a group, when approached, would take to the air in unison, while the observer was more than 100 yards distant. When these flights occurred in the neighborhood of the group area we could easily recognize the long call, flight, rapid flight and warning calls. The sex moan and copulation call were also sounded while the birds were in the air. The former occurred just before a gull landed near its nest and the latter while a male hovered above a female during an unsuccessful attempt to copulate. Later in the courting period the groups appeared to be more reluctant to leave the ground, and concerted flights were no longer seen. Incubating birds would remain over the eggs in an alert attitude even when the observer approached to within 75 feet of the nest.

The up flight and calls in response to danger in one group did not stimulate similar reactions in neighboring groups. If the observer slowly approached along a line approximately equidistant from two groups, one of these would leave the ground while the birds in the second group continued to stand on guard. If the observer now retreated, the latter would remain on the ground. The up flight, therefore, does not appear to be released either by calls or flight of any one bird. This was also indicated by the reaction of gulls which nested close together. Here the birds retreated individually and not as a flock or even in pairs. The concerted response of the group during the early breeding period, therefore, represented the simultaneous responses of individual members to the same external stimulus.

During the early part of June, when the colony as a whole reached the incubating stage, groups of birds could no longer be seen. A general survey of the distribution of nesting areas nevertheless indicated that this organization still existed. The nests were generally concentrated in the neighborhood of the areas previously occupied by the

groups. At Stone Harbor, the nests were situated along the banks of the streams on either side of these group areas (FIGURE 2), and on Muskeget Island they were radially distributed (FIGURE 1). However, a few exceptions were observed. These consisted of nests which showed a marked degree of isolation, and others which indicated an overlap of two adjacent groups.

Because the laughing gull rigidly exhibited a group organization throughout the season, it was interesting to speculate on the existence of a pecking order. All members of a group generally stood facing in one direction. Frequently, a bird would rise from the ground and seek to stand at the head of the group. Since little aggression resulted from these adjustments and because individual birds could not be followed for long periods, we were unable to determine whether these movements reflected a social hierarchy. The lack of experimental control also prohibited any conclusions regarding a pecking order in spite of the many aggressive contacts observed during feeding.

Sexual Behavior in the Group

The behavior of individual, sexually active, laughing gulls had its end in pair formation. The first sexual pattern to be recognized was the frequent use of the long call. Since this note was sounded during a wide variety of social situations, it could not be specifically correlated with any one. It was frequently heard in the apparent absence of specific environmental stimulus and also when it produced no reaction in other birds. Experimentally it has been demonstrated that the laughing gull must be at least physiologically conditioned before the long call of the breeding bird can be produced (Noble and Wurm, 1940a). In other instances, the long call appeared to stimulate similar activity in neighboring birds. In addition, this note apparently directed the next courtship pattern of the male toward calling birds. Males in the pairing charge posture were observed to run as much as 30 feet at birds producing the long call. The pairing and feeding charges, however, were also initiated in the absence of any preceding calls.

Sex recognition is based upon the fact that only males perform the charging behavior. Other birds at whom these activities were directed showed one of two types of responses. A male approached by another in the charging posture retreated for a considerable distance or completely deserted the group. Females reacted by holding their ground or retreating a short distance, then posturing erect, and flagging the head. The following note, taken on May 26, illustrates not only the

occurrence of the pairing charge but also the responses resulting in sex recognition:

Group of eight birds. One of three very erect birds (male) suddenly lowers head in pairing charge posture and runs at another, which responds by posturing erect and retreating one or two feet. The charging bird then charges two others which fly away. The male again charges the first bird which again postures erect and flags its head; is pecked in back or the head; does not retreat but continues posturing and flagging. After a number of such charges, the entire group has flown away except for the male and two females.

This type of behavior was usually followed by mutual erect posturing, head flagging, and eventually both members of the prospective pair reacted by food begging. It was difficult to recognize the definitive pair because more than one female sometimes responded to a single courting male. The opposite situation in which several males courted with a single female may be illustrated by the following field note:

In response to a male's feeding charge and sex moan, the female postures erect, flags head and food-begs three times. The male reacts only by posturing and flagging. This male is attacked by another male standing a few feet away. The same female displays similar behavior toward the second male. No nests in the area.

The following observation taken at another part of the colony shows how the pair was finally established:

Group of four gulls standing together (probably 2 males and 2 females). One male (δ 1) suddenly goes into the feeding charge posture, gives sex moan and charges at another bird (η 1) who postures erect and flags head. The male turns around and again performs feeding charge and gives sex moan. The female (η 1) begs. After two more beggings, η 1 is attacked by another bird (η 2, later found to be the true female mate of the charging male), and η 1 flies up in the air, circles once and again lands in the group. The attacking η 2 now postures before the moaning δ 1, and the fourth bird (δ 2) approaches and postures in front of the attacking η 2. The second male (δ 2) is now attacked by δ 1 (moaning male) and flies away. The remaining birds are now 2 females and 1 male. The male now uses the feeding charge and moan toward both females indiscriminately. η 1 seems to react willingly by posturing, flagging and three beggings; is now viciously attacked by η 2 and comes to rest about 6 feet from the resulting pair for about half an hour. In the interim the male continues to use feeding-charge at the remaining η 2 which, after almost 5 minutes of charging and moaning, begins to food-beg and is fed by the male. Courtship proceeded no farther.

While the birds were in the group, mutual food begging was never followed by food passing. At Stone Harbor we never observed birds in the group to copulate, but on Muskeget Island one such case was noted.

Sexual Behavior in the Pair

The definitive pair could be recognized when the birds deserted the group to occupy a nesting area. Apparently such desertions were initiated by the males, whose sex moan attracted the females to the selected site. This is evidenced by the following field note:

A new pair appears to be selecting a nesting site about 20 feet from the blind. This area was never occupied before. The male, just before landing, begins to moan; on landing assumes feeding charge posture and continues to moan. Female is circling about overhead, finally lands alongside male who is still moaning. Female goes into a frenzy of food begging. . . . After another flight by the pair, the female lands in the same place and about a minute later the male lands alongside reed pile on the opposite bank of the narrow stream. The male assumes the feeding charge posture and moans loudly. The female flies across stream alongside the male, postures erect and flags head.

We have observed two females attempting to nest with one male. When either of these courted with the male, one female attacked the other and drove it from the area.

Pairs of laughing gulls continued mutual head-flagging displays throughout the breeding season. However, normal copulation occurred only after a period of adjustment in which the basic behavior consisted of courtship feeding. Males were able to stimulate females to beg for food by using any of the following patterns: sex moan in the erect posture, sex moan in the feeding-charge posture, and fish-carrying together with the sex moan in the feeding-charge posture. Food begging was also initiated by either sex of the pair when no apparent stimulus from the mate preceded the activity.

Whereas no food passing was seen in the group, females were often fed by the male in the neighborhood of the nest. When food was carried in the beak, the male was always in the feeding-charge posture. Usually, however, the male regurgitated and the female snatched the offering either from his beak or the ground. Many instances were noted in which food was not passed in spite of persistent begging.

The importance of mutual food begging was revealed by the numerous instances of premature copulations. Males sometimes initiated this behavior with the head held high, changed to the copulation call but failed to mount because the female, instead of food begging, had retreated. In other instances, females vigorously dislodged their treading mates, but the males continued to sound the copulation call while fluttering in the air above the females. Early in the season many mountings did not lead to coition, apparently because the female failed to take the suitable copulatory posture. Such an incomplete copulation is illustrated by the following note:

The male returns to his mate and the strange female again approaches the pair but suddenly flies off. The male now gives a single food-begging call and head snap. The female also begs, with head much lower than the male, but not in posture typical of the female (copulation posture). The female walks beyond the male showing the back of her head, the male again food-begs with head very high, then changes to copulation call without wing flapping, although the female is about three feet away. The female now approaches and both birds give food-begging call but the female again begins to walk away. The male opens beak

wide and strains to regurgitate. The female turns back, with head low, begs twice, reaches in the male's mouth and takes out fish. The female starts to walk away and the male gives copulation call. The male suddenly runs up to the female, mounts, flaps wings and again sounds copulation call. For about a minute the female stands without reacting and the mounted male is in erect posture and calling. The male suddenly twice pecks the female on top of the head and she crouches. The male still has not tried to contact cloacas. During the time the male continues the copulation call, the female produces a rapid tossing of the head and then takes the typical female copulation posture.

Food begging by the female, together with the characteristic posture of mating, therefore, were necessary to copulation. No treading attempts occurred which were not preceded by food begging by the male. In this sex, the head tossing and *nnow* call used during food begging were smoothly transformed into the short downward pecking and *kaw-kaw-kaw* call of copulation.

The function of the latter call, and its associated head movement, was revealed in the above as well as the following observations:

Female is squatting on nest platform and male stands alongside, food begging. Male changes to copulation call and then back to food begging. Male mounts and gives copulation call and female rises, shows a rapid nervous shaking of the head which changes to food begging, head is turned around, gently pecks male's breast and attempts to grasp beak. Male pecks down about three times. Female is in low, food-begging attitude with body tilted forward. Male shows slight tail fanning, brings tail to left side and contacts cloacas. After dismounting, male postures erect.

The pecking movements of the treading male were not true pecks because the beak rarely touched the female's head. It is possible that they represent an incipient courtship feeding reaction, since the female also responded by soliciting food from the treading bird.

It was never definitely established that females invite copulation. We consider this probable, however, because begging females sometimes moved a foot or two away from the male and crouched motionless in a copulatory posture as though in anticipation of being mounted by the male.

Aberrant Sexual Behavior

Although the laughing gull showed distinctive methods of sex recognition and pair formation, variations from the normal procedure also occurred. The following observation made on May 29 illustrates that a strange male sometimes attempts forcibly to copulate with a female with which it is not mated:

A strange male lands near a squatting female and starts to food-beg with head high, then attempts to approach. The female starts to choke and the stranger steps back but continues to food-beg and again tries to approach. The female again chokes, gets up and starts to attack. Stranger retreats. As female turns to

go back to nest, stranger jumps on her and attempts to copulate. Female pecks at the male and dislodges him. Stranger hovers over female giving copulation call and attempts to mount. Another bird in the air dives at the stranger, attacks, and strange male retreats. The third bird is the resident male and lands alongside the female on the nest.

Goethe (1937) has made comparable observations and also recorded attempts at homosexual copulations in the herring gull. The following notes reveal the same behavior in the laughing gull.

Female squatting on nest (A) and male standing nearby. Suddenly male mounts female who then half rises from nest; male attempts coition and gives copulation call. Female food-begs, turns head around and grasps male's beak a number of times. In five attempts to contact cloacas, the male is twice successful. During all this time, a third bird (male) is standing about two feet away and for a long time appears indifferent. Suddenly the strange male starts a forward pecking motion of the head with closed beak (like a treading male) and then mounts the pair. All three birds are mounted now, strange male, resident male, resident female. All three hold this position for about half a minute and the middle male pecks up at the top male, then jumps with the strange male still mounted. They continue short fluttering flights with the top, strange male trying to remain mounted and the lower male struggling to dislodge him. Lower, resident male finally flies away and the upper, strange male returns to the female and mounts her. The male is giving copulation calls and the female does not object, but performs the normal beak and breast stroking of the male. Male finally dismounts without actually trying to contact cloacas.

The same strange male also tries to copulate with the male of another pair whose nest (B) is only five feet from that of the above-mentioned pair. The female of the pair is squatting on the nest and her mate stands alongside her. The strange male stands about five feet in rear of the pair. The sitting female suddenly gives two begging calls and head-snaps. The strange male mounts her male mate and attempts to copulate. Again there is the same fluttering fight with the upper male attempting to stay mounted and the lower male to dislodge him. The female is completely indifferent. The top male finally gives up the attempt and lands at its own nest (C) 25 feet away, occupied by a lone female. They are standing together quietly. The resident male returns to the female and nest (B); both stand quietly.

Since homosexual matings in free living birds are observed very infrequently and recognized only with great difficulty, the following note taken in another part of the Stone Harbor Colony is also presented:

Two birds, apparently males, are attempting to copulate with a third resident male which is courting its female mate. The mated male uses the feeding charge, moans, food-begs with head high, head-flaps and postures. Each time the bird charges its mate, one of the two strange males jumps on top of the resident male and attempts to copulate, at the same time giving the copulation call. One of the strange males actually mounts three times and the other mounts once. The resident male pecks the strangers and twice tosses the males over his head after grabbing them by the beak. The strangers finally fly away.

We believe this behavior indicates that the resident male was low in the social order of the group because it submitted to an attempted copulation and showed poor territory defense. This condition is further illustrated by the following note (taken soon afterwards) in which the low

head position during food begging showed that this same male did not completely dominate its female mate.

. . . The male starts food begging with head semi-erect and as the female also starts begging the male gives copulation call. However the male cannot mount because the female is begging with head very high. The male pecks at the ground twice, changes to food begging with reed in bill, drops reed and gives copulation call. This sequence is repeated eight times. Each time the male pecks down at the ground, the female starts to do the same, and the male rapidly approaches, gives copulation call, tries to mount but female retreats. . . .

Nesting Behavior

Since copulation always occurred away from the group area we assumed that it took place in the prospective nesting sites. Murray and Grey (1941) have seen pairs of laughing gulls copulating some 40 miles from the nearest known breeding ground. However, these observers did not indicate whether the observed locality possibly represented a new breeding ground. Prior to May 24 no nests were found at Stone Harbor, but the copulation sites were littered with masses of drift reeds. These apparently served as the platforms for the future nest. On the other hand, many birds preferred to assemble their own platforms. In such cases one bird, usually the male, would fly long distances with a reed held in the beak.

In the course of nest building, the sexes showed specialization of labor only in a general way. The males usually deposited the nesting material in small heaps in front of the incubating female. The male's industry was once demonstrated when a bird made 34 consecutive trips to a reed pile, scooped up some grass without landing and each time returned to the incubating mate. Sometimes, a male in search of nesting material robbed the nest site of another pair. In most cases, however, the laughing gull showed a distinct pattern of collecting material with many rapid pecks at the ground, thus filling the beak with short reeds. Except for the absence of the call, these pecking movements resembled the choking behavior.

Females were rarely observed gathering nesting material. They performed most of the nest building and incubation, although males also engaged in this activity. The nest platform was laid down haphazardly. The standing gull sometimes started to choke, picked up a reed, turned the head to either side and then dropped the reed along the shoulder. The major part of the nest was constructed in the following way: The squatting female picked reeds from the heap supplied by the male and built them into the nest by rapid pecking movements. At intervals the gull faced in a new direction, used the breast to mold the

nest, and continued building, with the result that the nest was evenly constructed.

In the Stone Harbor colony we discovered the last new nest, which as yet contained no eggs, on June 21. Nevertheless, many birds were still transporting nesting material. This activity apparently was confined to nests which did not hold complete sets of eggs. Nest building and egg laying therefore were concomitant activities. This was also indicated by the observations regarding "double nests." When examined on May 30, the southeast end of the main island contained 9 marked nests with dated eggs. When re-examined on June 15, dated eggs were found in only 2 of the marked nests, one of which was small. In addition, 7 other nests found alongside the original stakes appeared unusually tall. On closer examination, these were found to be "double nests" which could easily be separated into two. High tides and a bad storm apparently had washed the original eggs from these nests and when a new clutch was started, a second nest was built over the first.

Laughing gulls first revealed their nesting areas when birds used the choke behavior. At this time the pattern was performed between members of a pair as an exhaustive ceremony which led to nothing. Later, however, a bird would choke before settling on the nest, during nest building, while collecting nesting material, and before nest relief. Under these circumstances, the mantle feathers were generally smooth. Choking behavior was also used during aggressive displays which could easily be recognized by a marked erection of mantle feathers. The behavior was always initiated by the resident bird to prevent the approach of a stranger. In addition to observations under normal conditions, this was demonstrated by the following experiment:

A trapped bird is partially anesthetized (injected with 1.5 cc. of 3% evipal) to weaken fright reactions and loosely staked within three feet of a strange nest. Twenty-five minutes later, when the effect of the disturbance partly abates, the resident bird separates from the flying group and twice dives at the experimental bird attempting to peck it. The strange bird weakly flaps once or twice but generally stays quiet. The resident bird then lands on its nest, chokes a number of times, incubates and continues to choke intermittently for half an hour.

Territory was also defended by direct attacks. These occurred on the ground and in the air above the nest, showing that territory in the nesting laughing gull extended vertically, frequently to a distance of more than 20 feet. .

Noble and Lehmann (1940) have shown that broody laughing gulls are attracted more by the eggs than either the nest or nest site. Correlated with this is the fact that toward the end of June, when the incubation period was well under way, we noted that generally only one

bird stayed at the nest and incubated. When it was impossible for a laughing gull to brood its own nest and eggs, it sometimes reacted to a strange nest in a neighboring territory as shown in the following observation.

Chicken wire box balanced over nest containing three eggs. Resident gull returns to incubate and box is dropped trapping bird. Captive bird intermittently broods and tries to escape. About an hour later the mate returns, tries to reach the nest and eggs while the bird under the trap tries to escape. After a while the free gull goes to the neighboring nest, three feet away, and incubates. At intervals returns to its own area, is unable to incubate and returns to the strange nest to sit on the eggs.

Birds that were not occupied at the nest were either feeding or congregated in a flock in which no sexual behavior was shown.

On the other hand, laughing gulls exhibited a desire to incubate even when eggs were not visible. This was first shown when birds brooded a nest platform before eggs had been laid. Later the laughing gull would fly into the nesting area, land on top of its brooding mate and force it from the nest. Another method of nest relief was to "choke" in front of a brooding bird which eventually surrendered the nest. Males have indirectly developed a third method in which the sex moan is used. The brooding male responded to this call by leaving the nest to beg food from the male. No sooner were the eggs exposed than the male hurried to cover them.

DISCUSSION

The advantages of and justification for studying the social behavior of captive birds have already been advanced by Portielje (1930) and Steinbacher (1938). In a previous study of the black-crowned night heron (Noble, Wurm and Schmidt, 1938) it was shown that the behavior observed in the laboratory not only closely resembled that in the field but revealed the significance of some activities which would otherwise have been obscure. We have repeated this technique in the present study and can state that captive laughing gulls exhibited the identical behavior pattern of free birds.

The Group Area

In the laboratory cages the sexually active gulls as a group occupied a broad window sill or, when this was blocked off, an artificially illuminated platform raised four feet from the floor. Elevation and illumination therefore appear to be two requisites for the courtship area of the laughing gull group. Similar preferences are exhibited by *L. fuscus* (Richter, 1938). It was reported to the authors that in previous years

the laughing gulls in the Zoo appeared to concentrate on a broad circular mound. During 1940, we noted that this area was occupied by a variety of other birds. It was therefore not unusual to observe the gulls making mass excursions to the cage struts at a height of about 25 feet and there performing their pairing courtship.

In *L. atricilla* the area occupied by the group serves as a communal courtship ground. It is here that the initial bond of the pair is formed. It has always been assumed that when herring gulls arrive at the breeding ground they are already paired. According to Goethe (1937), herring gulls at this time select individual courting areas (*Standplatz*) in which they carry on their sexual behavior and near which they build their nest. Booy and Tinbergen (1937), however, described a social trysting place (*soos*) in which gulls attempted to court with a number of neighboring birds. Richter (1938) also described a "meeting place" for the herring, lesser black-backed and common gulls. In case of the herring gull, this author observed copulations to occur in this area which was occupied by birds in nuptial and winter plumage. In *L. fuscus*, Darling (1938) recognized a "headquarters" which serves as a communal courtship ground, while Kirkman (1937) noted the existence of group organization in the black-headed gull. Kirkman found that this condition was reflected in the manner in which the nests were distributed throughout the colony. *L. ridibundus* therefore very closely resembles the laughing gull in which we have observed a similar situation.

Darling (1938) has interpreted the existence of a communal courtship area as a means whereby the individual psychobiology of reproduction is reinforced by effective transmission of behavioral stimuli within the group. Richter (1939), however, pointed out that whereas *L. argentatus* and *L. fuscus* have recourse to a "meeting place," he could find no evidence for social stimulation of this type. In one pair in which the male fed the female after food begging, the latter was viciously attacked by its neighbors. Richter furthermore stated: "I believe that the attack on the eating female was not caused by food envy. . . . The attack continued for too long a time after the disappearance of the food and the attack was too clearly directed at the bird itself." (Translation)

Our own experiences with the laughing gull disprove Darling's interpretation, inasmuch as a variety of sexual behaviors (long call, sex moan, food begging and copulation) performed by one pair provoked aggression in neighboring birds. We believe that group organization

in a communal courtship area provides for frequent contact between many individuals that are in a like physiological state. Through specific reactions these individuals indicate their sex and permit the initial formation of pairs. Having done this, the laughing gull deserts the group to continue courtship and nesting with comparative degrees of privacy.

Territory

Davis (1940) reported that in the smooth-billed ani, territory defense is shown by a resident colony against strangers. Within the colony, however, there is no semblance of territory defense although aggression, in the nature of sexual fighting, does occur during the period of pair formation. The value of large colonies has been interpreted as providing greater chances of one bird meeting another in the same sexual phase. The same situation is reflected in the group area of the laughing gull in so far as we found no evidence for territory defense. It is interesting to note that Kirkman (1940) recognized two types of territorialism associated with defense of the nest, representing intra- and inter-group contacts. The group area of the laughing gull, however, is not a territory, but with certain minor differences resembles the "lek" of the ruff and blackcock (Heinroth, 1928).

The concept of territory as developed by Howard (1929) has been the subject of critical reviews (Nice, 1933; Friedmann, 1935; Lack and Lack, 1936). Recently Noble (1939) pointed out that different species of birds vary with respect to the role that territory plays in their social behavior. As a consequence, any definition that limits this phenomenon to sex, season or function, is inadequate (Meise, 1936).

The laughing gull exhibited a typical territory behavior when the pair selected a nesting site. A fixed region was defended by both sexes, though mostly by the male. In this species we were unable to establish that males and females show territory defense against their respective sex, although such behavior has been described in the herring gull (Booy and Tinbergen, 1937; Tinbergen, 1936b). Territorial residence is generally believed to endow a bird with greater success in combat (Noble, Wurm and Schmidt, 1938). Exceptions to this have been demonstrated in the case of the laughing gull, when a strange male succeeded in stealing nesting material from resident birds, and when a male entered the territory of a nesting pair and attempted to copulate with either sex. Roberts (1940) reported similar observations upon penguins in which strange males violated the territory of other birds in the presence of either sex. We are in agreement with this author's

suggestion that such observations point to the existence of a social order, and that social dominance in the laughing gull at least can sometimes overcome the fighting superiority associated with territory possession. This was also illustrated by preliminary experiments in which one of two nests, 25 feet apart, was moved to within 10 feet of the second. A short while later, both pairs attempted to return to their respective nests. The female of the translocated nest succeeded and soon busied herself incubating, while the male persistently attacked the other pair and prevented them from returning to their nest. This experiment as well as the observations of Noble and Lehrmann (1940) demonstrate that territory defense and residence are primarily concerned with the nest and eggs rather than the area *per se*.

Sex Recognition

The male flicker recognizes sex because the distinguishing secondary characters of other males stimulate aggression (Noble, 1936). In pigeons (Whitman, 1919) and song sparrows (Nice, 1937), the sex of a strange individual is determined by behavior and calls, and these also stimulate aggression in birds of the same sex. Laughing gulls form heterosexual pairs because males and females respond differently to the pairing charge. In the field, and more easily in the laboratory, it was recognized that this behavior pattern represented sexual fighting. During the first contacts, a charging male pecked a responsive female on back of the head, although she displayed by posturing erect. However, during subsequent contacts the male charged, suddenly stopped short of the posturing female, and vigorously preened on the breast.

Tinbergen (1936a) pointed out that in many animals sexual fighting occurs without any connection with territory, but no case of this had been adequately studied in birds. Although aggressiveness by the sexually active male laughing gull was first displayed within the communal courtship ground, the evidence against a territory drive was the fact that no definite part of this area was protected. In the laboratory, the male sometimes cleared the entire window sill of all birds. At other times only one end of the sill was defended. Lastly, after the male acquired a female, the pair deserted this area for a suitable nesting site. Another observation to indicate that masculine aggressiveness at this stage of the breeding period was neither the result of a territory nor dominance drive is the fact that only certain birds were regularly attacked while others were disregarded and permitted to stay in the area. In the laboratory the latter birds were either castrates or normal gulls that were late in showing sexual behavior and nuptial col-

orations. The exteroceptive stimulus for sexual aggression, therefore, appeared to consist of signs of sexual activity displayed by neighboring birds. This was clearly demonstrated when a male passed two or three birds, standing quietly, to charge at a more distant gull giving the long call.

We believe the herring gull resembles the laughing gull in this behavior, because Booy and Tinbergen (1937) clearly recognized that the long call, among other behavior patterns, provoked attack from other birds. Goethe (1937), furthermore, recorded the observation that "one female tried repeatedly to approach a male . . . was repeatedly bitten by him and nevertheless stood her ground." (Translation) The only other evidence in support of an aggressive pattern existing in *L. argentatus* is the statement by Steinbacher (1938) that birds at the nest ran at each other in an aggressive posture and moaned; and Portielje's (1928) observation of mock attack, in which case the male appeared to employ the choking posture. Steinbacher (1938) indicated that, in *L. fuscus*, pairs formed when the male used the equivalent of the moan and feeding charge posture. This pattern was alternated with choking activity but "without any apparent sequence."

Sexual Display

During a fight, sexually inactive laughing gulls frequently raised the head high and maintained a tall posture, facing the aggressor. This completely ended the encounter or temporarily saved the bird from being pecked. Similarly, gulls using the choke behavior for long periods, in defending territory, alternated by posturing erect a number of times. It appears therefore that the erect posture displayed by sexually active females has its origin in a defense reaction. During the breeding season this attitude is emphasized by displaying the back of the head with its black plumage and is then formalized into a head flagging ceremony in which the head is turned from side to side.

The typical gulls are recognized as falling into two groups, in one of which the head of the adult is white in summer while in the second the head is mainly black at this season (Knowlton, 1909). Dwight (1925), however, divided the gulls into four groups in which nuptial pigmentation ranged from white through gray, brown and black. We believe that *L. ridibundus*, an example of a brown-headed gull in which the hood is also comparatively small, represents one of the transitional forms between the main groups. This intermediate position, is also reflected in the manner in which the bird displays.

On the one hand, we recognized that the laughing gull exhibits a

courtship ceremony in which the nuptial black coloration of the head is employed in a distinctive "head-flagging" ceremony. On the other hand, the Australian silver gull, a white-headed species, does not show this behavior pattern. Instead, its equivalent is expressed in another display pattern employing the black wing tips. Portielje (1928) and Goethe (1937) recognized "erect posturing" in the herring gull, but made no mention of a behavior resembling the "head flagging" of *L. atricilla*. Portielje also described the following behavior: "Two males are walking around in the courting place among other birds giving themselves airs in their best and most effective manner . . . a female approaches . . . as one male goes away the female grasps and pulls him by the tips of his wings or tail." (Translation) Marples and Marples' (1934) excellent description of posturing in terns also emphasized a specialized use of the black wing tips in display. Different species of Laridae therefore employ courses of movements in which the black plumage is exhibited to the greatest advantage.

The "upward display" of *L. ridibundus* described by Kirkman (1937) matches in every detail the "erect posturing" of the laughing gull. The characteristic "head flagging" of the latter appears to be absent in *L. ridibundus*, although Kirkman described a display in a pair of gulls in which, ". . . they inclined their heads sometimes to right, sometimes to left, and occasionally the male would turn and incline his head to the prospect behind." This author, nevertheless, "never saw so elaborate a display again," which in this instance probably involved an exceptional use of "a sudden jerk of the beak to one side," which accompanies erect posturing. We never observed "head flagging" in a black-headed gull in the New York Zoological Garden, although this bird engaged in active courtship with a number of laughing gulls. Instead, this gull frequently displayed by posturing erect, facing away from its mate and rapidly running in semicircles about the bird. This apparently served to display the black wing tips because Kirkman observed tail pulling in this species.

The most comprehensive treatment of the taxonomic relations between gulls has been made by Dwight (1925). However, the phylogenetic classification of these birds has been generally avoided because of the difficulty of determining which of the characters used in distinguishing species is the more primitive. Comparative studies of bird behavior have yielded, according to Tinbergen (1939), the following significant generalizations:

" . . . Conspicuous and highly specialized structures, whose par-

ticipation in nonsocial processes cannot be found, have a social, communicative value."

"Signal movements may occur without morphological structures but not vice versa."

". . . special inborn behavior elements are probably more constant in evolution than many morphological structures" and "the ceremony is older than its organ."

Phylogenetically the intermediate nature of the nuptial plumage of the black-headed gull represents either the acquisition or the loss of a distinctive structure. Since the ceremony employing this structure appears to be absent, it follows that the hood, characteristic of this species, does not represent an evolutionary acquisition of a specialized structure. This interpretation, furthermore, is consonant with the facts; namely, that vestigial structures may be without demonstrable function and that present-day gulls evolved from a dusky ancestral form (Maschtaler, 1940).

Courtship Feeding

Courtship feeding has recently been the subject of a comprehensive review by Lack (1940), who stated that birds are the only vertebrates exhibiting this activity. Since fish and reptiles do not feed the young, it appears probable that courtship feeding developed simultaneously with the avian type of parental behavior. Behavior of the laughing gull indicated that courtship feeding may be regarded as a forward projection of parental behavior to the period of sexual activity. We found that chicks and sexually active adult females displayed the same pattern in food begging, and that one of the stimuli for this activity, in either case, is the male's sex moan. Terns also exhibit courtship feeding and Marples and Marples (1934) described a situation in which a male presented fish to the female, who in turn passed it on to the chicks. At Muskeget, we once observed a similar performance on the part of the laughing gull.

Wachs (1933) believed that courtship feeding supplied the female with extra nourishment needed for egg laying. Observations on captive herring gulls convinced Steinbacher (1938) that males feed receptive females as a substitute for copulation. However, we are in agreement with Lack (1940) that "the main function of courtship feeding is clearly display." The feeding charge, during which the male laughing gull carries fish, indicates that fish presentation can be projected to a stage in the breeding cycle in which the pair has not definitely formed. Apparently one of the functions of this behavior is the maintenance of

the initial bond of the pair. The sexual nature of this activity is further indicated by the fact that males occasionally feed females in the absence of any object which could provide a stimulus for parental behavior.

(Goethe (1937) reported that *L. argentatus* infrequently shows food-begging behavior during the winter. We have observed the same reaction in *L. atricilla*. It seems best, however, to distinguish winter food begging from similar behavior of sexually active birds because the former occurs exclusively in the presence of food or play objects while the latter is frequently displayed in the absence of these stimuli. We have elicited sexual food begging in the laughing gull by the injection of sex hormones (Noble and Wurm, 1940a). On the basis of these experiments, we concluded that androgens stimulate the courtship feeding behavior of the male and that estrogens control the feminine type of food begging with low head.

In the laughing gull, the respective roles of the sexes during courtship feeding are rigidly maintained. These are obviously physiologically controlled. However, Plath (cited in Lack, 1940) and Hampe (1940) observed that homosexually mated female parrots sometimes pass food to their mates. Although courtship feeding does not occur in the black-crowned night heron, Noble and Wurm (1941) reported an aberrant type of food begging between paired adults. Apparently the guttural voice in one member of a pair stimulated food begging in the sexually inactive mate regardless of sex.

Sexual Dominance and Copulation

Two aspects of sexual behavior in the gull, the "pairing charge" and "food begging," are referable to sexual dominance. The pairing charge shows the male to be the dominant bird because the female, though severely pecked, does not attempt to peck back. This behavior also illustrates the influence of threat and aggressive display during the period of sex recognition.

Lack (1940) interpreted the billing exhibited by several species of birds as incipient courtship feeding, but he regarded the same behavior in herons as representative of passing nesting material. However, billing in the black-crowned night heron has been shown to be a dominance gesture first evidenced by nestlings in competition over food supplied by the parent (Noble, Wurm and Schmidt, 1938). In this sense, billing in the night heron and food begging in the laughing gull are analogous indicators of dominance developed in relation to food. During

normal food begging, the female calls and tosses the head like the male, but the head and the body are held very low. If, during this activity, the female's head is raised higher than that of the male, the female is again severely pecked, but she does not attempt to peek back. Mutual food begging between sexually active laughing gulls therefore serves to keep the male and female in the dominant and subordinate positions respectively. This interpretation of courtship display has also been advanced by Friedmann (1934) and Portielje (1936).

In the case of pigeons, females of homosexual pairs take the masculine attitude during copulation; and such cases have been assumed to indicate the "relative masculinity" within the pair (Whitman, 1919). However, Noble, Wurm and Schmidt (1938) stated that mounting behavior in the night heron depends upon the relative dominance between members of a pair. The similarity of these explanations has been experimentally demonstrated in the case of the domestic chicken by Allee, Collias and Lutherman (1939), Hamilton (1938), and Hamilton and Golden (1939); in the ringdove by Bennett (1940); and in the night heron by Noble and Wurm (1940b). The subordinate food begging posture of the female laughing gull, in which the body is tilted forward in a semicrouch, head retracted and tail inclined, serves to accommodate a treading male. Similarly the mounting movements of the male are facilitated by the food-begging attitude of the female. Treadings which are not followed by coition can be explained on the grounds that the female is not completely subordinated and therefore does not take a suitable copulatory posture. Aberrant mounting behavior in penguins also reflects an inadequate dominant-subordinate relation (Roberts, 1940). It appears, therefore, that cases of incomplete copulations are to be explained not with the assumption that the female has not reached the proper stage in the breeding cycle (Howard, 1929), but rather as indicating that one bird has not secured sexual dominance over its mate. This explanation is especially applicable in the case of a strange male gull mounting but failing to copulate with a resident female that had already started to lay eggs.

SUMMARY

The laughing gull colony is subdivided into a number of distinct groups during the breeding season. The area occupied by each group serves as a communal courtship ground in which pairs form, and is also the center about which nests are distributed according to restrictions of the environment. Nests are located in relatively dense vegetation and

this is correlated with the presence of comparatively small nesting territories. Territory defense is carried out on the ground and in the air.

The communal courtship area is adapted to provide the maximum number of contacts between many birds that are in an optimal physiological state for reproduction. The first social contact between sexually active gulls is stimulated by the long call. Sex recognition is mediated by a behavior pattern, essentially aggressive in character, which is initiated by the male and to which males and females react differently.

The initial bond of the pair is reinforced by courtship display and courtship feeding which continue throughout the incubation period. Successful copulations occur only after the male secures sexual dominance over the female. This dominant-subordinate relationship is under physiological control and expressed by "charging" and "food-begging" behavior.

A preliminary account of the sexual behavior of the Australian silver gull, *L. nova-hollandiae*, indicates that this species employs behavior patterns that closely resemble those of the laughing gull. Fundamentally the same behavior patterns appear to exist throughout the Laridae.

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EXPERIMENTAL MODIFICATION AND CONTROL
OF MOLTS AND CHANGES OF COAT-COLOR
IN WEASELS BY CONTROLLED LIGHTING*

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FIGURE 1 (GRAPH); PLATES 1-7

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* Aided by grants from the Penrose Fund of the American Philosophical Society, 1938-1939, and the American Academy of Arts and Sciences, 1942.

† Publication made possible by grants from the income of the Centennial Endowment Fund and the George Herbert Sherwood Memorial Fund.

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INTRODUCTION

Changes in pelts of ferrets and fitch (Bissonnette, 1935) and of mink (Bissonnette and Wilson, 1939) have been found to be conditioned by changes in the duration of daily exposures to light, irrespective of environmental temperature and color of surroundings. Pelt-changes were controlled for all the ferrets studied and for most of the mink. The failure of some of the mink originally studied to molt and assume prime winter pelt in summer and early autumn, as the result of reduced daily lighting, was undoubtedly due to irregularity of the lighting schedule and failure to adhere to it. In subsequent trials, in which a proper schedule was adhered to, all animals whose lighting was reduced molted and grew winter pelts (Bissonnette and Wilson, unpublished data). It is therefore certain that pelting time for both ferrets or fitch and mink can be controlled by properly controlled daily illumination.

Both ferrets and mink make their autumn as well as their spring change of pelt by complete shedding of the previous coats, not by any changes in the hairs already grown, as has been supposed to take place in animals that whiten in winter. But they are not good species in which to follow the courses of the spring and autumn changes of pelt, because neither of them undergoes a sufficiently marked change in color by which such course can be followed easily without much handling of the animals. A suitable small mammal for such studies is found among the weasels, of which some species change to white or near white each winter and others only to a lighter shade of brown. In those that whiten, it is easy to follow the course of the wave of color-change over the body and note its progress without handling the animals (PLATES 1-7).

A study has therefore been made, beginning in May, 1939, and lasting to March, 1943, of two species of weasel available in New England and Pennsylvania, to see if their pelt-cycles can be light-controlled. These are the large New York and long-tailed weasel (*Mustela frenata noveboracensis* (Emmons)) and the smaller short-tailed or Bonaparte's weasel (*M. cicognanii cicognanii* Bonaparte). One specimen was an abnormal male used with the controls in the first part of the study and as an experimental during the last year and a half. At first, he was thought to be a least weasel (*M. rixosa* (Bangs)), because of his small size and short tail with only a few short black hairs at its tip, but, later, he was properly identified by the late Dr. Glover M. Allen. Apparently, he controlled his own light-cycles. The vagaries of his molts

and color-changes on normal days have been described by Bissonnette (1942).

Previous studies pertinent to this problem have reported somewhat conflicting results and conclusions for various animals.

Miyazaki (1934-1935) pointed out, in the case of the Japanese mejiro bird or white eye (*Zosterops palpebrosa japonica*), that lengthening days bring on sexual activity and shortening days stop it and induce molting. If daily lighting is increased during the molting period, re-feathering becomes irregular and abnormal.

Witschi (1935) found that the cyclic changes of plumage in the weaver finch (*Pyromelana franciscana*) depend upon cyclic hypophyseal activity and that these changes were the same at Iowa City, Iowa, as they were in Africa. He concluded that altered environmental conditions, including lighting, do not affect their plumage-cycles.

Walton (1937) caused mallard drakes (*Anas platyrhynchos*) to take on eclipse plumage in February and March, instead of at the normal time in June and July, by subjecting them to artificial lighting in addition to periods of normal light. Complete castration did not prevent this change of plumage in the first year, but did in the second. It was concluded that eclipse plumage is not caused directly by a testicular hormone acting on the feather follicle, but that removal of the testes sooner or later affects some other endocrine organ or organs which consequently become insensitive to seasonal change (light) or fail to produce the hormones that cause eclipse.

Witschi and Miller (1938) found that injections of sex hormones failed to affect the plumage of European starlings (*Sturnus vulgaris vulgaris*). This appears to indicate that their plumage-cycles are independent of the gonads.

Salomonsen (1939) described the plumage and color-changes of the rock ptarmigan (*Lagopus mutus* (Montin)), and pelt-changes of stoats, weasels and varying hares—based on observations of ptarmigan in nature, as well as museum specimens and skins in correlation with average temperatures at regions and dates of capture. From these data, without experimental studies, it was concluded that the change to low temperatures conditions and controls the change of white coat or plumage in these mammals and birds; the varying susceptibility to this change among the different species determining the time and degree of whitening. Reduced illumination was dismissed as of minor or no importance. The course of change in the mammals was not described in detail.

Brown and Rollo (1940), in the region of Chicago, Illinois, experi-

mentally controlled the daily lighting of whydahs and weavers (*Steganura paradisea*, *Pyromelana franciscana*, and *Vidua principalis*). They found that, in that latitude, the first two species do not come into nuptial plumage until two years of age. But, by subjecting them to sixteen hours of light a day, all three species were brought into nuptial plumage in their first year, with *Pyromelana* most resistant, and *Steganura* least resistant to light treatment. When exposed to sixteen-hour light-periods, a *Pyromelana* remained in nuptial plumage for more than a year, while a control, under natural daylight conditions, went into eclipse plumage in December, slightly more than a month after the experiment began. A male *Pyromelana*, one year old, under restricted daylight periods, and in process of molting from eclipse to eclipse plumage, very soon began to regenerate nuptial feathers when subjected to sixteen-hour light-periods. The type of feather regenerated in a male *Pyromelana* in nuptial plumage, after plucking, was affected by duration and intensity of daily light-periods. This is inconsistent with Witschi's findings, made at approximately the same latitudes.

Burger (1941) found that molting and subsequent refeathering of the European starling (*Sturnus vulgaris*) will occur irrespective of day-length; but a reduction of day-length favors or speeds up these processes. Their inherent rhythm is modifiable by altered lighting. This agrees with Witschi, so far as inherent rhythm is concerned, but shows, in addition, that the course of the rhythm can be modified by altered lighting.

Leshner and Kendeigh (1941), in experiments with captive birds during two winters, found that *Zonotrichia albicollis* (white-throated sparrow) and *Colinus virginianus* (bob-white), normally with a prenuptial molt in spring, will molt out of season when the daily photo-period is raised to fifteen hours. *Passer domesticus* (European house sparrow) and *Junco hiemalis* (snow bird), normally without prenuptial molt, failed to do this. *Zonotrichia*, *Passer*, and *Colinus*, normally undergo a complete postnuptial molt in late summer, but molted similarly in early spring after their photo-period was reduced from fifteen to nine hours. This was reduced either immediately, or at the rate of fifteen minutes a day, or five minutes a day. The time that the molt began and the rate of loss of feathers was accelerated during the more rapid rates of decrease of the photo-period. The total time (65-73 days) required to renew the plumage was about the same, irrespective of the rate of decrease of the photo-period or of the rate of feather-loss. The weight of the birds increased during the longer photo-periods and decreased when feathers were being lost and renewed. This points to

the length of the daily light-period as a controlling factor in the times of molts in birds having them either in spring or in later summer, or both; but it is not capable of inducing molts under conditions of day-length in which the birds normally do not molt. Species differences come into play here. The tendency to molt or not to molt within a given day-length is a specific inherited character.

Rollo and Domm (1942) tested the optimum day-lengths and intensity of illumination conditioning the change-over from eclipse to nuptial plumage in weaver finches (*Pyromelana franciscana*). They found that 14-hour day-lengths caused the earliest change-over and completion of the nuptial plumage. The responses of birds on other day-lengths, shorter or longer, were in direct proportion to the length of daily light-period above and below this optimum. In utilizing foot-candles varying from 3.5 to 350, used for fourteen hours a day, the optimum intensity was 126 foot-candles. Birds on that intensity responded five to six weeks ahead of those subjected to other intensities. At low and high intensities no eclipse feathers were regenerated. These weaver finches can be brought into full nuptial plumage in ten weeks when subjected to 14-hour daily light-periods of 126 foot-candles intensity. Anything above or below the optimum causes regression.

Lyman (1942), for varying hares (*Lepus americanus*), has found that a reduced daily period of illumination causes molting of the brown coat and growth of white hair in autumn, and an increased daily light-period induces molting of a white and growth of a brown coat in spring. Either of these changes of coat-color can be induced, out of the normal season, by appropriate lighting schedules well adhered to. He found a short period of prewinter physiological condition occurred, which induced a minor molt and a change from brown to brown, before the major molt from brown to white. His studies were made upon plucked areas on the shoulders and so did not follow the course or wave of change over the animal as a whole, or the effects of quickly reversed changes in lighting. He found that castrated or thyroidectomized hares responded like normal animals to light manipulation. So it would appear that neither the sex glands nor the thyroids are essential to the color-changes or molts.

Per Höst (1942), from his studies of controlled lighting with the willow ptarmigan (*Lagopus lagopus*), has learned that changes of daily lighting induce changes of plumage color in that species, irrespective of high or low temperatures. He caused birds to whiten out of normal season and to return to normal spring plumage and lay eggs in winter, by appropriate lighting schedules. The birds went through their series of

molts or altered them, even skipping some, depending on the type of lighting regime.

Miss Rothschild (1942) found that, though she could not initiate or induce molts in the stoat or English ermine (*Mustela erminea*) with changes of temperature, she could hasten completion of the winter molt and growth of white fur by lowered temperature, causing the change to white to be completed in seventy hours. Not all stoats became white in winter under similar conditions of temperature, which seemed also to control the whiteness of the coat. She has described the course and order of whitening of parts of the animal and has pointed out that both autumn and spring color-changes are due to complete molts and growth of new hairs. The autumn whitening is not due to bleaching of summer hairs as was supposed by some authors.

The experimental studies of Brown and Rollo, Burger, Lyman, and Höst do not support Salomonsen's contentions that change of temperature is the inducing factor in these seasonal molts; nor do those of Bissonnette, and Bissonnette and Wilson. Although Miss Rothschild found that cold was a factor in speeding up the molt in autumn with regrowth of white pelage, she agrees that the induction or initiation of the molt is not dependent upon low temperatures preceding such initiation. She suggests that the longer time taken in spring to assume the brown coat may be due to the more gradual change toward warmth in spring, and says it is "almost irresistible to associate this with the relative suddenness of winter snowfall and the more gradual disappearance of snow in the spring thaw."

As will be seen in what follows, our experiments on the short-tailed or Bonaparte's weasel give results in agreement with those of Lyman and Höst, and with those of Bissonnette, and Bissonnette and Wilson, on nearly related species, but are contrary to the conclusions of Salomonsen. The New York weasel agrees with the Bonaparte's weasel in the times and causes of molts but normally fails to become white in this locality (Hartford, Connecticut), under the experimental light-control operating in these experiments. We have not tried the effects of cold upon the speed of the autumn molt for fear of losing our animals from chilling, because our studies were planned to continue for a period of three and one-half years with varied procedures, and we had so few animals to begin with.

Reported Pelt-changes of Weasels

In Connecticut, Bonaparte's weasel is said to turn completely white in winter except for the black tip of its tail, which remains black. The

New York weasel is said merely to molt its dark brown summer pelt and replace it with denser fur of a lighter brown or of a yellowish tinge. Farther north, in the Boreal zone, and in parts of northern New York State, about fifty per cent of the latter species turn white, and there is said to be a sex difference, in that males fail to turn white while females do (Goodwin, 1935; Hamilton, 1933).

These facts indicate, in two closely related species, a marked and specific difference in response to change, in whatever factor or factors condition the molt from summer to winter pelage and back again. The agent or agents appear to act quantitatively to condition a difference in the amount of pigment in the seasonal pelts of the two species, the difference being more extreme in one species than in the other. The response of the New York weasel in the Connecticut region is intermediate between that of the mink and ferret or fitch, with respect to change of texture and density of fur, but not of color, as compared with that of Bonaparte's weasel, which undergoes complete or almost complete change of color, so as to produce a protective camouflage in regions of snowy winters. The different responses of the two species here, and the greater response of the New York weasel farther north, indicate differing responses to differing amounts of change of the same factor or factors in the environment, acting more strongly farther north. Bonaparte's weasel exhibits a greater susceptibility to stimulation or inhibition by increase or decrease of the operating factor. This may be change of temperature (Salomonsen, 1939), or change of color of surroundings to white and back again (as commonly held by trappers and others), or change of duration and/or intensity of available daily illumination, as in ferrets and mink (Bissonnette, 1935; Bissonnette and Wilson, 1939), or as in varying hares (Lyman, 1942) or willow ptarmigan (Höst, 1942). The Bonaparte's weasel responds with change of both texture and color to a smaller or slower change in the effective factor or factors. Under like conditions, the New York weasel changes only texture and shade of brown; but, with more marked environmental change farther north, in about half the cases it also makes the double change of texture and color. However, this may be in part due to local or physiological races.

Of the New York weasels farther north, those changing to white in winter are females (Hamilton, 1933) while those remaining brown are males. In our laboratory experiences, the males are the more venturesome and spend more time by day outside the dens. We are led to suspect, therefore, that the males are less affected than the females, as they are exposed to daylight for longer periods daily, while the latter

stay in the dens for a longer time and are thus less exposed to light. Hence, length of light-exposure rather than temperature changes or the sight of snow are determinative. For, though the males are more exposed to cold and snow, nevertheless, they remain brown, which is contrary to expectation if the temperature and visual environment were causal factors. On the other hand, the females, in cold weather, have their periods of illumination reduced. This is consistent with the illumination explanation favored by us.

On the working hypothesis that change of illumination is the factor concerned in these reactions, the time and rate of change of daily lighting were altered experimentally to see if that would change the time and rate of change of pelt in both species of weasel, in Connecticut, under like conditions of temperature and dark-colored surroundings, using animals all from the same Pennsylvania locality for the experiment.

Both the controls and the experimental animals were kept in the same room with unpainted red brick walls, dark-colored trim, and dark cement floors, under temperature conditions, not rigidly controlled, but practically alike, relatively cool, and showing little or no seasonal variations, except greater daily fluctuation in winter when the heating system of the building was operating (graph, FIGURE 1; PLATE 7). During the summer months, when there was no artificial heat, an approximate mean between minimum and maximum daily temperature was indicated. The mean of its fluctuations was much more nearly uniform throughout the year and showed little seasonal variation as compared with the outside temperatures. It was the same for both controls and experimental animals, except when the controls were put into a warmer room while the experimentals were given light in addition to normal daylight; so that, if raised temperature tended to induce brown color, it would act against the reduced light of the controls, if light is also a factor. Aside from this, temperature-changes and changes of color of surroundings were not experimental factors in causing the differing responses of experimental and control animals. These have been the two most commonly credited with controlling the pelt color-changes in such animals (graph, FIGURE 1).

Much was learned from an initial experiment in 1937 that came to nothing because of deaths due to improper cages and the inability of weasels to survive on rations that had nevertheless proved successful with ferrets. Two weasels should not be kept together in small quarters for long, because one will kill the other and eat it. The animals are too quick to be handled easily with thick gloves, without injury,

and cannot be kept in cages with hinged or sliding doors that must be opened to put in food and water or to clean the cages. So a new type of cage was adapted, patterned on those used by mink breeders. This has proved very successful after minor changes, made since it was described elsewhere (Bissonnette and Bailey, 1940; PLATE 7).

MATERIALS AND METHODS

Eleven lively, uninjured weasels were secured from a trapper in Pennsylvania (Nos. 14 ♀ B, 16 ♀ N.Y., 7 ♂ N.Y., 11 ♂ N.Y., on May 23; 2 ♂ B, 5 ♀ N.Y., 6 ♂ N.Y., on May 30; 17 ♂ N.Y., 13 ♀ B, 18 ♂ N.Y., 20 ♂ N.Y., on June 13, 1939). Those marked "B" were judged to be Bonaparte's weasels on the basis of size and length of tail and black tail-tip. Those marked N. Y. were identified as New York weasels. Seven were judged to be males on the same basis and superficial observation, and four to be females. None was handled for sexing for fear of injury.

They were kept in similar individual units of den and runway adapted for this study (Bissonnette and Bailey, 1940). This permitted observation of the animals at will, and easy interchange of runways and dens for cleaning. The dens provided darkened seclusion, with some light entering around the edges of the covers. Two groups of five units were fastened together temporarily on racks, moved easily on large casters, to act as experimentals and controls. This arrangement facilitated cleaning, disinfecting, and sunning parts at regular intervals and obviated injury to the animals by handling them in changing quarters (PLATE 7).

Milk and water were supplied to each animal in amounts just sufficient for each day, using glass drinking vessels, protected with brass pipes having openings above those in the glass. This protection prevented the animals from breaking the glasses and swallowing glass fragments, as some had previously done, with fatal results. Two small wire springs, one near the top, the other near the bottom, kept the vessels in place; the lower one preventing the animals from pushing out the glass tube and escaping into the room, as happened when the lower spring was not in place. A wire nail from top to bottom of the inner end of the brass tube was added, as double insurance, to divide its lumen and prevent animals from getting through the tube. No escapes occurred after all these precautions were taken.

A tendency to gnaw the wooden boxes was stopped by covering the wood with wire or metal, after one animal died from swallowing long slivers of wood. Frequent inspections had to be made to prevent ani-

mals from spreading the wires and escaping, because, at first, they were very persistent in such attempts. They gave it up after three or more months, but occasionally one tried it again. This trial period at first was shortened for a later acquisition of specimens by placing a glass under the wire cover of the dens so the animals could not reach the wires to separate them. After a week or so, the glass was removed, and the animals no longer bothered with the wires.

They were fed 35-40 cc. of whole milk, unpasteurized, to which a pinch of brewers' yeast and one of Cal-Cod, fish-oil concentrate, were added each day. The quantity of food was determined for each animal by giving no more than the amount used each day. This was also true of the ration of liver, given twice each week, and, on other days, lean meat or a bird or mammal carcass and entrails, thus providing for vitamins. The meat was placed on top of the wire runway to be taken at leisure. Each received a living mouse or two each week. The same care was given to all.

Five animals (Nos. 11 ♂ N.Y., 16 ♀ N.Y., 7 ♂ N.Y., 17 ♂ N.Y., and 14 ♀ B) were used as experimentals and the other six as controls (Nos. 5 ♀ N.Y., 6 ♂ N.Y., 18 ♂ N.Y., 2 ♂ B., 13 ♀ B., and 20 ♂ N.Y.). By day, the experimentals and the first five controls were arranged in units placed on two racks equidistant from, and directly in front of three windows, at a line ruled upon the floor, to keep them uniform and to insure equal temperatures at all times, and equal luminous intensities during daylight time for both sets of animals. The other control (No. 20 ♂ N.Y.) was placed for a while at the back of the room in much less intense light by day. After control No. 6 ♂ N.Y. died on October 10, 1939, it was replaced in the front line by this animal.

During the first part of the experiment, when the effects of light-reduction were being tested, the controls remained at the same position on the line, day and night. Experimentals were moved about five feet forward into a "dark room," just large enough to accommodate cages and rack during the hours of darkness specified (graph, FIGURE 1; PLATE 7). The entrance to this "dark room" was curtained, so as to be light-tight, by a quadruple curtain of dark blue flannelette. Also, curtains for the sides permitted air to enter slowly to prevent suffocation of the animals. It is probable, however, that the temperatures around the animals in this "dark room" rose slightly above those around the controls. The room was not heated except that, as fans drew off air through window vents by day, it was replaced by warmed air from the halls of the building. If, therefore, falling temperatures are the cause of molting in weasels to lighter color or white pelt and denser fur, the controls

should molt first, changing to a greater degree than in the case of experimentals. The only light color visible to the animals was the white painted lining of their dens and other cages between them and the windows, which were soon dulled by use. Little or nothing resembling snow could be seen by the animals. Any light-colored surroundings were alike for both groups and thus were eliminated as factors in the experimental procedure.

Experimental light-reduction was begun on May 23, 1939, with the experimentals received at that time, while others were added to both controls and experimentals as they came in. Experimentals were placed in the "dark room" for the periods shown in the graph (FIGURE 1). This also shows the relation of their light-time to that normal for Hartford, Connecticut, in such a way that their daily light-time, on any date, can be compared with the time of year when that day-length was normal. Since, under natural conditions, these animals spend much of their days in their dens and forage both day and night, we cannot know how much exposure to light each one actually gets from day to day. For this reason, the lighting controlled was merely that of naturally available light. Available light-time per day was reduced for experimental animals on July 21 by one-half hour each *five* days to a seven-hour period, and on September 23 directly to six hours. It was then left at that level until November 12. Thus the "reduced-lighting" first part of the experiment was constituted (graph, FIGURE 1).

Light-time was then increased by one-half hour each *four* days, beginning on November 13, 1939, in order to produce a sharper increase in daily duration of light than usually occurs in spring. This increase was continued at the same rate, when the experimental day-length reached that normal for the season, by adding electric light after dark (FIGURE 1). At this time, the controls were moved to the next room, away from the added light. On normal days, they were kept in the basement room until April, 1941, when they began to be used as experimentals. The other room was warmer than the experimental room. Hence, the controls were warmer than the experimentals in the cold room with its artificially lengthened days. In the case of the controls, therefore, this should have prevented, or, at least, have slowed the whitening of the coat; or it should have hastened its return to the brown phase, if rising temperature is to be considered a factor in bringing on the summer coloration.

Light-hours available per day for experimentals reached twenty on February 28, 1940, and then, on March 2, the period was reduced by one-half hour each *four* days to a minimum of one hour per day. This

was continued from July 29 to August 1, by reducing added lighting and using the "dark room" after normal day-length was passed. Increase followed again at the same rate to twenty hours per day, from December 28, 1940, to January 6, 1941, when the experimentals were returned to normal days of about nine hours and thirteen minutes. In this way, they were subjected to gradual but rather rapid changes in duration of available daily light twice between May 23, 1939 and January 6, 1941. Controls on normal light-cycles were available at all times for comparison with experimental animals (FIGURE 1).

Gonads and accessory sex organs of animals from both groups were studied whenever individuals died, as in the case of seven individuals. One of the seven was a male New York weasel sent us by Dr. Enders of Swarthmore College in the summer of 1942, which died within four weeks, showing a heavy infestation with mites and lice.

The experiments, with variations, were repeated using three of the former controls (Nos. 13 ♀ B, 2 ♂ B, and 5 ♀ N.Y.) as experimentals, and the surviving former experimental (14 ♀ B) and a former control (20 ♂ N.Y.) as controls, beginning on April 12, 1941. No. 14 ♀ B had been returned to normal days on January 6, 1941, after being returned to almost complete summer pelt by December 21, 1940. When the controls were changed to a reduced lighting on April 12, they were well along toward the summer pelt, but not yet completely changed. Their schedules of light-time are shown in FIGURE 1.

A comparison of the dates at which animals changed pelts in relation to the time of change of lighting, the number of available hours of light for each, and the dates when such light-hours would be normal, etc., show how quickly and completely the weasels respond to change of day-length, and the relation of these changes to daily illumination. All this can be read from the graph covering the three and one-half years of the experiment and, in addition, the relation to temperature in the experimental room for the year (summer, 1941 to summer, 1942), with maximum and minimum daily outside temperatures for the same period. It may be added that the temperatures during the rest of the experimental period did not differ significantly from those shown in the graph, and these differences were not related to the results of the experimental treatments.

After April 12, 1941, the new experimentals were subjected to daily lighting, reduced one-half hour each four days to a minimum of one hour per day on July 13-16. This was then increased at the same rate to nineteen hours, on December 4-7; then at the rate of one hour each four days to 24 hours, on December 24-31; then decreased, one

hour each four days, to fifteen hours on February 3-6. Next they were reduced directly to normal days of ten hours and 35/100, from February 7 to March 10; and to four hours per day, March 11-24; then to one hour, from March 25 to April 14; and, finally, increased directly to normal days of thirteen and three-tenths hours of light (FIGURE 1). The former control, 14 ♀ B, was kept to the schedule of normal days during this time, together with No. 20 ♂ N.Y. There was no experimental modification of daily light-cycles for either group of animals thereafter. Both remained on normal days until November 19, 1942, when 13 ♀ B and 14 ♀ B became white together, showing return to normal pelt-cycles.

OBSERVATIONS AND RESULTS

The series of photographs of control 13 ♀ B and experimental 14 ♀ B, both Bonaparte's weasels, up to April 12, 1941, and of control 14 ♀ B and experimental 13 ♀ B, from that date onward, shows the type and times of changes of pelts for each (PLATES 1-7). In the graph (FIGURE 1), the vertical short lines crossing the lines of light schedules with the X and a year number (39, 40, 41, or 42) mark the date of a photograph of 13 ♀ B. The circled X marks the dates of photographs of 14 ♀ B, throughout the experiment. The date of the photograph is inscribed upon it with the number and sex of the animal (PLATES 1-7). The weasel marked September 26 in PLATE 1 represents a normal summer weasel or control on that date.

First Change to Winter Pelt

(PLATE 1)

On or before September 15, 1939, experimental 14 ♀ B began to show definite signs of changing its pelt. Loose brown fur was rubbed out from the feet, the under side of the tail, upward on the sides of the legs, and laterally up the sides of the body. Short white hairs began to show in these regions, which assumed a gray color. Then a ring of white hairs appeared around the tail, immediately in front of the black tip, and another at its base near the hips. White spread gradually up the tail and legs, the sides of the body and hips, and around the neck, from below upward. A white spot then appeared behind each eye, and the upper lips became gray, then white. When a band down the back about an inch wide was still brown and the tail all white except its black tip, a white spot appeared upon the bridge of the nose and the ears became white inside and out (PLATE 1). The white nose spot

spread to meet the white cheeks and lips and upward between the ears. This condition was reached between October 24 and 31 (PLATE 1).

Almost synchronously with the Bonaparte's weasel, just described, the New York males, Nos. 11 and 17, rubbed out the old summer dark brown fur, in the same manner and pattern, and replaced it with new winter fur of a little lighter or grayer shade. A little later the experimental 16 ♀ N.Y. followed with similar changes.

Replacement of brown fur by white on 14 ♀ B stopped on or about November 15, before she became completely white, but still retained some brown on the hips and on the nape of the neck, and also a brown spot between the ears and a few brown hairs on the shoulders (PLATE 1). The New York weasels finished the change to light-brown winter pelt, 11 ♂ N.Y. and 16 ♂ N.Y., on November 20, and 7 ♂ N.Y., on the 22nd, except for small spots on top of the head and between the shoulders, when the animal died, probably from swallowing glass. These spots are often slow to change (Hamilton, 1933). Weasel No. 17 ♂ N.Y. died on November 6 from swallowing long splinters of wood gnawed from the nestbox and perhaps some glass also. Summer fur had not yet been shed from his back and neck, but his sides were in new winter pelt. He was about half through his molt and replacement of pelt. These were all experimental animals. In all the New York weasels the change of pelt followed the same course as in Bonaparte's weasels with light brown instead of white.

The controls behaved as follows:—On or about October 15, 13 ♀ B began to pass through changes similar to those of 14 ♀ B; and, before the 31st, reached the condition shown by 14 ♀ B on or about October 1 (PLATE 1). She failed to become completely white, but remained a "gray-back" throughout the winter, from November 27 onward. She was marked with a band of gray about three-quarters of an inch wide, extending from between the ears to the base of the tail, during this period.

The abnormal male, 2 ♂ B, failed to change coat in October and November with the other controls, but began to assume white hairs between January 10 and 26, 1940, in spite of being moved with the other controls into a warmer room each night for some time previously. He reached his maximum whitening about February 6, and held to this amount of white until between March 12–25, when a brown band appeared along the flank and sides from the neck to the lateral surfaces of the hind legs, and spread a little way up the sides. A white saddle and a white area around the ears and across the base of his tail and hips remained through the spring and summer and until January, 1941. Then

he molted again and assumed a brown pelt, although the temperature in the room was not raised at this time nor for some time previously, nor was his light-ration increased. As discussed elsewhere (Bissonnette, 1942), he seemed to control his own light-cycle by changing his habits as to time spent in the lighted runway per day, as compared with time in the dark den. It is suggested that he induced a modified pelt-cycle in this way. His change to white and back again stopped far short of those achieved by either 13 ♀ B or 14 ♂ B, control or experimental, in their first winter. He began to assume summer brown fur in the spring of 1940, at the same time as the other controls, but followed the manner and pattern of the experimental 14 ♀ B, which was induced, by increased lighting, to begin this change in December and January, instead of the normal time, about March 12 or later. His change of pelt stopped after proceeding only a little way and hairs ceased to fall out or new hairs to appear.

Control 6 ♂ N.Y. died on October 10, 1939, without change of pelt, and was replaced in the front line by 20 ♂ N.Y., from the back of the room. All the remaining control New York weasels began to change pelt about the same time as control 13 ♀ B, but they merely replaced the hairs shed with others of lighter shade of brown on the body, and their feet became white. The patterns of replacement varied somewhat with the individual, but none showed any white on the body, tail, or upper parts of the limbs, as did those described by Hamilton (1933). The patterns were as variable, but we observed only lighter brown in areas described by him as white in his New York weasels from New York State. Ours were from Pennsylvania. There may well be a local race difference in this respect as there appears to be between the weasels of the same species (*M. frenata*) taken in Berkeley, California, where all remain brown through the winter, and in Salt Lake City, Utah, where all or very nearly all turn white in winter. Transfers from Berkeley to Lake Tahoe, in the Sierra Nevada, remain brown over winter in that locality, while transfers to Berkeley from there and from Salt Lake City turn white in winter, as they did in their native haunts (Professor E. Raymond Hall, in personal letters). All the controls retained their winter pelts and colors until the spring molt began, after March 1.

Return to Summer Pelt

(PLATES 1-2)

For experimentals, increase of daily light began on November 13, 1939, passed normal day-length on December 10, and reached twenty hours of light per day on February 28 to March 2, 1940.

Experimental 14 ♀ B began to show return to brown before December 28, when she was receiving light-periods equal to those of March 16 in nature (PLATE 1). This contrasts with February 23–March 22, in Ithaca, New York, for the normal beginning of spring molt (Hamilton, 1933) or that of control 13 ♀ B, about March 12–25, exposed to normal light in the same room. The increase in light-periods for 14 ♀ B was more rapid than in nature, yet she kept up well with the normals in fur change during the corresponding daily period, both in nature and in the case of the control animals.

Return to brown coat was first indicated by graying of the lips around the vibrissae, presence of short brown hairs next the skin among the longer white ones on the body (PLATE 1), and some spreading of the small brown patches on the hips and shoulders. A brown strip across the bridge of the nose soon spread upward to the crown and laterally around the eyes. This brown underfur can be seen through the white before it shows in photographs. A brown band, without white hairs, then appeared along the flank and side of the body from neck to tail and spread upward toward the back, leaving a white band along the ridge, which narrowed to about three-quarters of an inch in width, extending from the base of the tail to the ears. This was divided into two strips on the neck by a narrow strip of brown remaining ever since the first taking on of white coat. This brown is apparently due to persistence of brown hairs from the previous summer's coat. The animal had, at this time, parts of three seasons' coats on his body simultaneously. The crown was also brown between the ears. The animal resembled a male Bonaparte's weasel shown by Hamilton (1933; figure 1, plate 9) for March 29. Further spread led to a brown face with white brow-band, white sides of the neck, and browning of the tail from rump to tip, except for a white ring next the black tip and another at the base (PLATES 1, 2), reached about January 26, 1940, and carried through the summer until about August 25, or earlier. The same light-periods led to almost synchronous molts in 16 ♀ N.Y., although the precise limits of the replacements were not plain, as in the 14 ♀ B, and barely show in photographs.

Control 13 ♀ B underwent no change from her "gray-back" condition, assumed in November, until after March 12 (PLATES 1, 2), and New York controls behaved likewise. Timing of molts in the two species was closely approximate both in nature and with experimental lighting. They differed only in the whitening of whole body except tail-tip, in the one; and whitening only of feet, with lighter brown of body, in the other.

Between March 12 and mid-May, control 13 ♀ B resumed summer brown pelt, in the manner most usual for such change, and not like that of 14 ♀ B; namely, by the appearance of dark brown hairs, first along the ridge of the back, spreading laterally down the sides to the limbs and feet, and over the face much like 14 ♀ B (PLATES 1, 2). Before June 6, she was brown except for some gray on the toes. She remained in this condition through the summer and until October 14 (PLATE 2), after which time she began to molt and assume white fur again.

Second Winter Pelt, 1940-1941

(PLATES 2-3)

Between August 14 and 25th, experimental 14 ♀ B began to shed brown hairs and put on white short fur. As before, the whitening spread up the feet and legs, along the tail from the underside and tip toward the upper side and base, from the lips up the face and around the neck, from ventral to dorsal parts. Before September 26, she was all white, except between the ears, a narrow strip mid-dorsally along the neck to the shoulders, and tail-tip, which did not change in any of our weasels. She became completely white, except tail-tip, before October 6 and remained white through November 20 (PLATES 2, 3).

It is noteworthy that she became completely white before October 6, 1940, after her daily light-ration had been gradually reduced to one hour per day, July 29-August 1, from a maximum of twenty hours on March 1. In the autumn of 1939, she failed to reach a complete white coat after her available light was reduced to six hours per day, September 24-November 12. Her greatest whitening was reached about November 15, at the much slower rate of decrease of daily light, and from a high point of only thirteen hours in May. The two years differed in (1) time of beginning light reduction, (2) rapidity of reduction, (3) low point of daily light-ration, and (4) high point of daily light-ration.

Control 13 ♀ B, in contrast, remained in summer brown until after October 14, 1940 (PLATES 2, 3). At some time in late October, she began to replace brown hairs with white, proceeding in the usual manner from toes toward the mid-line of the back, and from the underside and base of the tail to the tip. This was about two-thirds complete on November 4; almost complete on November 9; and finished to pure white before November 20 (PLATE 3).

From this last date until about the end of November, both experimental and control female Bonaparte's weasels were in complete white winter

pelage, while 2♂B differed from both. Control New York weasels were slightly later and slower to make their change to winter pelt.

Resumption of Summer Pelt, 1940-1941

(PLATES 3, 4)

Between November 20 and December 7, experimental 14♀B began to get gray along the back, over the nose, around the lips, brow and crown. This time, her browning progressed in the usual, most common manner from mid-dorsal line laterally and ventrally down the sides, tail and limbs; not as in her previous change to brown in January, 1940. Before the 13th, she was brown about halfway down the sides and almost to the upper parts of the limbs. This browning progressed quite rapidly until she was in almost complete summer brown pelage, about December 25. She failed to become brown on the lower parts of the legs; and a narrow white ring around the tail, next the black tip, persisted through March 10 (PLATE 3). This shows that she stopped her spring molt before completing it, as in the previous year; but much nearer completion than before. This was, doubtless, in part, due to her being returned to normal day-lengths on January 6, involving a reduction of daily light-periods from twenty hours, immediately, to nine hours and thirteen minutes, on that date.

Control 13♀B began to return to brown summer coat before March 10, 1941, after remaining white from November 20 (PLATES 3, 4). She also followed the usual method of browning from mid-dorsal line ventrally.

Change to Winter White, April, 1941

(PLATES 3, 4)

During the last week of March, 1941, 14♀B, whose daily light-periods had been reduced to normal on January 6 (from twenty hours to nine hours and thirteen minutes), with normal daily increments thereafter, began to replace her brown hair with white, up the limbs, sides, and along the tail. She was in the same room as control 13♀B, and they had been on the same lighting schedule from January 6 to April 12, while occupying adjacent cages on the same rack, with no modification of normal day-length for either animal, and under similar conditions of temperature for the preceding two seasons. Yet, in April, 14♀B changed to white winter coat while 13♀B, former control, was changing to summer brown coat. The change to white was plain on April 9. Whitening continued until about April 24 (PLATE 4), when only a small patch of brown remained between the ears of 14♀B, while

13 ♀ B retained white only on the posterior quarter of the tail, fronts and backs of the legs, and lower parts of the flanks. Weasel 14 ♀ B remained in this white coat with brown spot between the ears until she molted from it into complete white in late December. She had stopped her molt again, and hairs of brown became tight again, before completion. As before, hairs from coats of two seasons were present on her at the same time. Apparently, the gradually increasing periods of daylight finally stopped the change to white "winter" fur in April, a time when such change to white never occurs in nature. This all occurred under relatively small changes of temperature, within narrow limits. Control 13 ♀ B, under exactly similar conditions and variations of temperature, simultaneously, or nearly so, changed from white to brown.

Incompleteness of change of the control animal to brown, and to white in the first winter, and of experimental to white and to brown, indicate that the change-over in either direction can be stopped short of completion by either gradual or sudden change of the daily period of available light, or by early reversal of increase or decrease of such periods. The exact dating of the reduction of daily light for 14 ♀ B on January 6, 1941, and the time of inception of her molt to white in the last week of March, give a measure of the latent period for such response or of the time required for adjustment of her internal reaction-system between the time of the beginning of the stimulus and the time of visible reaction, as shown by growth of white hairs and loss of brown.

The Reversed Experiments

(PLATES 4-6)

On normal days from January 6, 1941, 14 ♀ B passed that summer fully white, as stated above, except for a small brown spot between her ears, and molted this coat completely and became entirely white before December 24, 1941. She remained in the normal full winter white pelt until after March 9, 1942, when she turned brown, in the usual manner, from mid-dorsal line to legs, but remained white on the forelegs and feet, under the ears, hind feet and in a ring next to black tail-tip. She just failed to reach full summer brown coat on normal days this time (PLATES 5, 6).

The lighting periods of 13 ♀ B were experimentally reduced by one-half hour each four days, from normal days on April 12, 1941, to one hour per day, July 13-16, followed by an increase at the same rate to nineteen hours on December 7, and by one hour each four days to twenty-four hours a day on December 24-31. Under these conditions, she con-

tinued to turn brown until some time between April 24 and May 16, and remained in brown summer coat until between October 10 and 16 (PLATE 4). She then began to turn white in the usual manner, reaching complete white pelt on November 6 (PLATE 4), just 48 days before 14 ♀ B, on normal days, did so. Before January 20, 1942, she was turning brown again in the usual manner (PLATE 5) and, on March 18, reached full brown, except for white on tail, feet and throat, when 14 ♀ B, on normal days, was showing first signs of turning brown, which she completed, also with white feet and ring on tail, about May 6, about 49 days later (PLATE 6). Let us continue with 13 ♀ B. Her lighting had been reduced at the rate of one hour each four days, from January 5, 1942, to February 6, and then directly increased to normal days of approximately ten hours and six minutes, rising normally to about eleven hours and twenty-four minutes on March 10; then cut to four hours, and again to one hour per day, on March 24–April 10; then returned to normal days of about thirteen hours. On April 25, 1942, she showed unmistakable signs of changing to white pelt (PLATE 5). She continued to change in the regular manner, until she stopped, all white, except for brown on the top of her head and nape and back of neck about May 27 (PLATE 6). She remained in this condition until only about June 27, when she began to take on brown coat-color along the sides, but not from the back downward (PLATE 6). She retained white in a band about three-quarters of an inch to an inch wide along the back from brow to base of tail, except for the brown crown and nape of neck, and a band of white, about an inch wide, around the tail next to the black tip (PLATE 6). But after July 28, the change to brown stopped. Retention of this white would seem to indicate that the follicles in this region were not yet through with the growth of white hairs before the internal reaction-system took on the growth stimulation for brown hairs, and that they were refractory to this change and remained white without molting or growing new brown hairs.

On or before October 29, 1942, both 14 ♀ B and 13 ♂ B began to change over to white coat, with white showing through the brown fur along the sides, flanks, legs, and underside of tail, and below and behind the ears. By November 4, this white began to show through on the bridge of the nose, around the vibrissae of the lips and along the tail, three-quarters of the length from the black tip toward the body (PLATE 6). By the 8th, the tails of both animals were white almost to the body, and white was halfway up the sides and over the head to the crown.

Very little brown or gray remained on either animal on the 13th (PLATE 7), and, on the 19th of November, both were completely white,

reaching that condition on the same day, within twelve hours of each other, remaining in this condition until March 12, 1943, when 14B began to turn brown, followed later on (March 30) by 13B. On normal light-cycles their molts had become synchronized after all the changes induced by the manipulated light-cycles, but, by the next spring, their own habits, on normal light-cycles, had made a difference.

DISCUSSION AND CONCLUSIONS

The following general facts are revealed by this study. Animals making a change to white appear to assume white hairs always from belly, chest and throat upward toward the back; on the tail, from under to upper side, and from the permanent black tip toward the base. These hairs form a more or less definite pattern over the face and head, the last part to become white being a strip broadest between the ears and narrowing posteriorly down the nape of the neck to the shoulders or farther. This change may be stopped short of completion by these last parts remaining brown or gray. New York weasels follow a similar order of molt and change of pelt almost synchronously with the Bonaparte's weasels; but only their feet and legs and perhaps the sides of their necks and edges of their flanks turn white or gray, the rest changing to a lighter shade of brown.

The brown coat is usually assumed by growth of new hairs, browning from mid-line of the back ventrally to the belly, breast and throat, and down the tail from base to the black tip, and down the legs toward the feet, which may or may not become brown. New York weasels change back to darker brown in the same order. But, if return to longer days becomes effective before all dorsally placed hair follicles have completed their growth of white or lighter hairs, and, if such change of day-length is rapid enough, the loss of white hairs and growth of brown may occur along the sides of the body and leave the dorsal parts white, until a succeeding molt and color-change occur.

It, therefore, becomes evident that molts and changes of color, both to white and to brown, can be stopped short of completion by appropriate artificial or natural manipulation of light-cycles, as was done in this experiment, or by the abnormal Bonaparte weasel 2♂B, himself (Bissonnette, 1942). The change-over, either to white or to brown, may be induced in these weasels at any time of year if allowance is made for a latent period of adjustment of the internal reaction-system of about three to three and a half months after effective change of light-cycle is made. Either gradual or sudden and immediate large reductions or increases in day-length are effective in inducing molt and

change of color, and the greater the degree of change of day-length, the greater the final change to white or brown or in the shade of brown over the body.

These changes of pelt-color can be stopped at different stages short of completion, or can be taken to completion by appropriate light-control, either experimentally or by the animal itself, by change of daily habits. This latter change may be made in nature as a response to cold or warmth by the animals' remaining more or less time in the dens and dark, or in the light in the open, or by hunting by day or by night. It is suggested also that local races of the same species may differ genetically in degree of response to changing light-cycles, as the abnormal 2 ♂ B differed from the apparently normal 13 ♀ B and 14 ♀ B used in this study. In addition, the apparent sex difference in response among northern New York weasels may be partly due to different habits of the two sexes in relation to winter cold, or to hunting by day or by night in winter, with females in the dens most of the daylight hours and males more venturesome by day.

After various modifications of time, rate and degree of color-change induced by light-manipulation, the animals can and do return to normal color cycles, normally synchronized with the seasons, provided such return is not made in an abnormal manner (as was done to both 14 ♀ B and 13 ♀ B when they were first returned to normal days). Their last change to white in November, 1942, was a normal change, and they came into white coat-color within twelve hours of each other. Return to normal light-cycle from longer days induces molt and whitening, even in spring, as was shown by both of these animals. Return from shorter days induced molt and browning in varying degree, as was shown by 13 ♀ B in July, 1942, even before completion of the previous growth of other-colored hair on parts of the body.

Temperature-change was not a causative factor in any of the changes of pelt in these experiments, although, as was pointed out above, it was not entirely ruled out as a possible modifier or even an indirect agent acting by keeping animals in nature in their dark warmer dens during cold weather and thus giving the major or direct factor of altered lighting its chance to be effective. In every case, when controls and experimentals differed, the higher temperature would have worked against the results attained.

The possible effect of seeing snow in the environment in bringing on the white winter coat was ruled out, since all animals were in the same room with dark floors and walls, and any light-colored objects visible to them were there throughout all the seasons, and for both experi-

mentals and controls. In nature, however, snow might also act like cold to keep animals in dark dens in winter or autumn and so indirectly reduce their lighting.

Our results lead us to conclusions disagreeing with those of Salomonson (1939) regarding the factor inducing white coat in weasels, and in accord with those of Bissonnette (1935), Bissonnette and Wilson (1939), Höst (1942), Lyman (1942), Leshner and Kendeigh (1941), Brown and Rollo (1940), Miyazaki (1934-1935), and Burger (1941), to the effect that altered daily period of illumination is the factor directly inducing the seasonal molts and changes of coat-color of weasels and probably of the other birds and mammals studied. Other suggested factors, such as cold and snowy surroundings, may indirectly be factors in this reaction by acting through altered periods of exposure to light.

Miss Rothschild's findings are in agreement with ours in so far as she finds that altered temperatures do not induce molting. We have not tested the possible effects of cold on rapidity of molt and regrowth of hair, once they have been started by other means. We agree with her that color changes in these animals involve complete loss of hair, as well as growth of new hair. We also saw signs of a little bleaching of the old hairs before they dropped out in autumn; though even in the New York weasels, that changed to lighter brown. This, however, was not the cause of the lighter coat, which consisted of new hairs of lighter shade. We also found variability, as between animals of the same species under the same conditions, and from year to year in the same animals, but no carry-over effect from one year to the next. Some animals changed from brown to brown in autumn, and from white to white; from brown to white in spring, and from brown to brown. So molts need not alternate in color. Also, animals may have hairs from three different seasons at the same time, as the results of incomplete molts from both colors.

While it is not believed that all animals are alike in respect to the factors conditioning and controlling molts and color-changes, or in respect to their reactions to these factors, it is concluded from the above results that, for the weasels studied, change of color and molting are photo-periodic responses, synchronizing the changes of pelt and color with the seasons in a generally adaptive manner, with different results in different species, and in local races of the same species, produced by natural selection upon originally variable species. That these adaptations to seasonal changes of environment are not yet completely effective is shown by the differences in reactions of 2♂B and 13♀B and 14♀B, from the same locality in Pennsylvania, under similar con-

ditions in these experiments. The animal also retains some control, albeit unconscious, over its reactions in this respect by its habits in relation to daily periods of illumination. The same animal, in nature, need not change pelt or color at the same time and to the same degree in succeeding years, because other factors in his environment, such as scarcity of food, cold, or depth of snow, may alter, from season to season, the time needed to be spent in the open or in the den, and thus control the daily period of illumination at the time of change of pelt, so as to induce molting, with or without change of color of new hair, at an earlier or later time in the year. This can account for the various conditions in which weasels, and presumably other species reacting similarly, are to be found at the same time in similar or different localities.

Lyman's findings (1942) that castrated and thyroidectomized hares undergo cycles of color-change like entire animals would tend to show that Bissonnette's (1935, 1942) suggestion, that the thyroid is probably concerned in the color-changes of such animals, is probably not well-founded. The fact that hypophysectomy abolished the cyclic molting and regrowth of hair in ferrets (Bissonnette, 1935) indicates that this gland is involved in the reaction. Bissonnette's findings, that the differences in time and amount of whitening of different body regions of the little male weasel (1942) indicate differences in degree or rate of response to the endocrine factor or factors mediating the color change, are given added emphasis by this study. There is added also the observation that apparently there is a time factor in allowing the hair follicles in late-whitening regions to complete the growth of new hairs of the previously induced color, before they respond with molting and growth of other-colored hairs. This is shown in the "gray-back," the animals with brown remaining on head and neck, the animals becoming only partly brown, and leaving white on the dorsal regions, with failure to molt these regions, when other parts of the body change color by molt and regrowth of hairs. Some parts require higher concentrations of the internal factors or hormones to be affected, or take longer to respond to the acting factor than others, and are refractory to changes in the concentrations, or in the hormones, for some time longer than other regions or follicles. Whitening or browning do not follow the all-or-none method of reaction for the animal as a whole.

There is a latent period before the change in lighting, either by increase or by decrease, becomes translated into molting and regrowth of hair of either color. Whether this is shortened by a greater degree or rapidity of change is not very evident from these results. It is

shown that some parts of the body surface, as well as the follicles therein, fail to respond at all, if the change comes too soon after a previously acting change. Also, an animal may change from white to white, and brown to brown, without alternation of color phases. So the previous reaction of the follicle does not control the one being initiated, or influence it appreciably. Control of this must be outside the follicle, probably in the pituitary (Bissonnette, 1935).

SUMMARY

1.—From May 23, 1939, through December 1, 1942, two normal and one abnormal Bonaparte's weasels, along with eight New York weasels (later reduced by deaths to three), were subjected to normal daily light-cycles as controls as well as to variously modified experimental lighting schedules, under conditions of temperature, care, and dark-colored surroundings as nearly identical as possible. When temperature conditions had to be different, the difference was such as to act against the lighting schedules, if both were factors in control of molts and color-changes in these animals.

2.—For experimental animals, daily periods of light were reduced, either gradually or suddenly, by the use of an appropriate dark room, and increased gradually, at different rates, and then suddenly, by reducing the dark-room time and adding electric light after nightfall, between the limits of one hour per day and twenty-four hours.

3.—Temperatures in the experimental room where all the animals, both controls and experimentals, were kept for most of the time, were recorded on a Bristol's recording thermometer and compared with minimum and maximum daily temperatures, for more than one and a half years, on a graph of the years covered. Alterations from normal for this locality were not great, and were in the direction of the mean in summer, and above the maximum in winter. Temperature change was not an appreciable factor in the observed modifications of pelt and color-cycles in these animals, nor of time and degree of molting. Temperature-cycles are not in control of molting or color-changes in these weasels.

4.—Reducing the daily periods of light induced molting and regrowth of new fur, in varying degrees, for both species of weasel. In the Bonaparte weasels, white replaced brown, and in New York weasels, lighter brown replaced dark brown on the body, and some white appeared on the feet. The black tail-tips remained black throughout.

5.—Increasing daily light-periods caused molting and change to dark brown, in varying degrees, over the bodies in both species.

6.—Incomplete molts in both directions (toward white or toward brown) were produced as a result of early reversal of increase or decrease of daily light-time. This could be controlled so as to leave the animal for some months with parts of its body showing hairs from each of three consecutive growths of hair.

7.—Even on normal days, molts in either direction are not always complete, and "gray-backs," in winter, or animals with some white in places normally going brown, occur in summer, among the controls. The "gray-backs" also occur in nature and, probably, so do cases of incomplete summer browning. These are quite easily understood if daily light-periods control molting and change of color.

8.—A latent period or adjustment-period intervenes between the time of increase or decrease of light-ration and the shedding and regrowth of hair. This appears to be about three months, more or less. Whether it is amenable to experimental modification of its duration is not yet clear.

9.—Not all animals of the same species react alike, even if secured from the same locality, and the same animals react differently in different seasons or years. Local races, in which a majority react alike, are probably produced by natural selection so that, in regions with snowy winters, a majority turn white in autumn, and continue to do so after removal to regions where there is little or no snow in winter; while a majority remain brown in winter, in localities where snow is scarce, and continue to grow brown winter coats when removed to regions with winter snow. Studies on this problem should, therefore, be made on animals known to react alike because they belong to the same local race. In such a case the locality where experiments are carried out is not important.

10.—After light-manipulation and consequent modified color-cycles lasting through two years and several color-changes, animals return to normal pelt-cycles, if returned to normal light-cycles in a normal direction or manner, but not if the return is abnormal—i.e., too abrupt, or in an unusual direction. Return to normal day periods from longer days induced whitening in the Bonaparte's weasel and lighter brown in the New York weasels. A return, however, from shorter days, caused a change to dark brown, in spring and summer.

11.—That this stimulus is received through the eyes and acts through the anterior pituitary gland is indicated from Bissonnette's studies on ferrets, a nearly related animal. That the thyroids and sex-glands are not essential is at least suggested, if not indicated, by Lyman's (1942) study on the varying hare.

12.—These results and conclusions are in agreement with those of Höst, Brown and Rollo, Leshner and Kendeigh, Miyazaki, Walton, and Burger, based on studies on birds, and suggest that this causal control of similar phenomena is quite widely distributed among birds and mammals. But that light is invariably the controlling factor in such changes is by no means yet shown. As with sexual periodicity, other factors may operate in other animals to bring about the adaptive synchronization with the seasons. Reductions of temperature or changes in any other factor or factors that drive the animals underground, or into dark dens for longer periods, may indirectly induce the taking on of white or lighter coats in autumn, and the reverse may induce return to darker or more adaptively colored coats in spring. Changed habits of the animals in this regard may appear to be self-imposed and bring about unusual times and degrees of molting and change of coat-color.

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EXPLANATION OF PLATES

This series of photographs follows the changes of coat-color of two specimens of Bonaparte's weasel (*Mustela cicognanii cicognanii* Bonaparte) under normal seasonal conditions of daylight and also under experimental lengthening and shortening of light exposure artificially produced in the laboratory. The observations extended from May, 1939 to March, 1943. The photographs here reproduced are dated September 26, 1939 to March 12, 1943. Both weasels were females, and are designated in the plates as 13 B and 14 B. The experiments with these and other weasels are recorded in detail in the text.

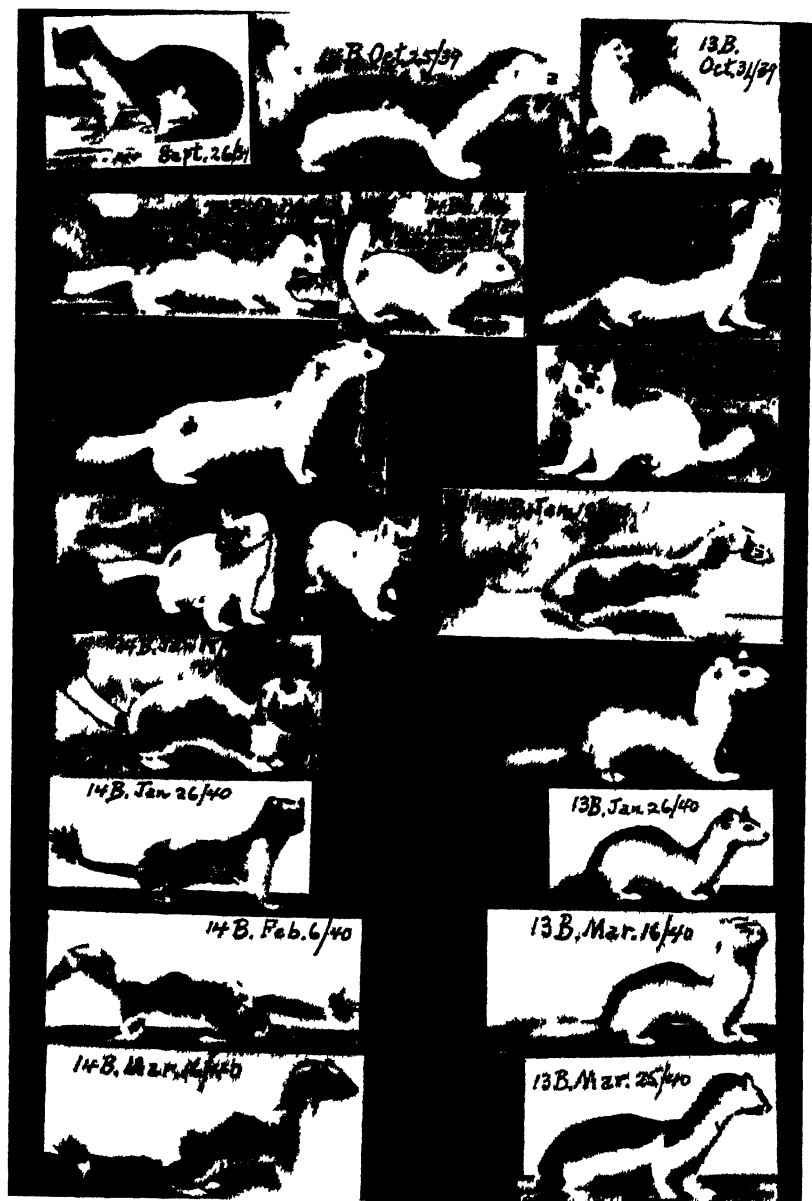
Weasel 13 B acted as control until April 12, 1941, while weasel 14 B was under experimental conditions during the same period. Beginning with April 12, the experiment was reversed, weasel 14 B becoming the control, and weasel 13 B the experimental animal. The captions accompanying the successive plates comment on the progress of the experiments.

PLATE 1

FIRST CHANGE TO WINTER PELT AND BEGINNING OF SUMMER
PELT

(September 26, 1939, to March 25, 1940)

The photograph marked "Sept 26, '39" represents a weasel with normal summer coat, as of that date. The control (13 B) and the experimental specimen (14 B) both changed normally to the nearly white condition shown in the photographs of both animals dated Oct 31, '39. Control 13 B retained a gray back throughout the winter. 14 B was exposed to artificially increased daily light beginning with Nov 13, 1939, finally reaching 20 hours of light daily, from February 28, 1939, to March 2, 1940. She began to put on a summer brown, out of season, before December 28, 1939, and developed progressively a mottled brown coat, as shown in the photographs marked Jan 9, Jan 18, Jan 26, Feb 6, and Mar 16. Meanwhile, the control (13 B) remained white, with a gray back, until March 16. By March 25, she showed rapid change toward the summer pelt.



BISSENIITE AND BAILEY CONTROL OF CHANGES IN COAT COLOR



BISSONNETTE AND BAILEY CONTROL OF CHANGES IN COAT COLOR

PLATE 2

RETURN OF FIRST SUMMER PELT COMPLETED AND BEGINNING
OF SECOND WINTER PELT

(April 10, 1940, to November 4, 1940)

Between March 12 and June 6, 1940 the control (13 B) assumed the full normal summer pelt, which remained until October 14, when it began to change to the second winter pelt in normal fashion. This was about two-thirds complete by November 4.

Meanwhile, 14 B carried through her summer pelt until August 14, as shown in the plate. She had her daily light-ration reduced experimentally from 20 hours (March 1) to 1 hour daily (August 1).

Between August 14 and 25, she began to shed her summer coat and put on a winter coat, becoming much whiter than the control, according to the photograph of September 11, and reaching complete whiteness, except the tail-tip, before October 6 (see also photograph marked Nov. 4).

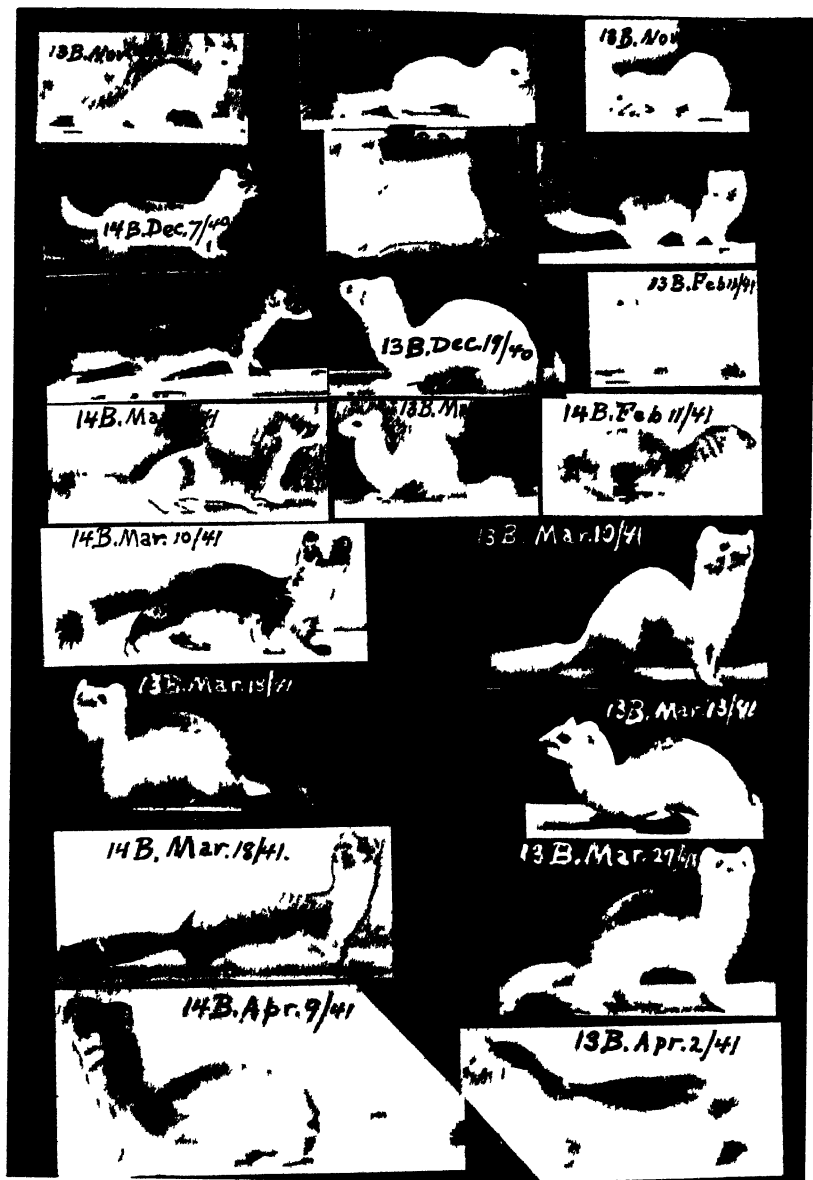
PLATE 3

SECOND WINTER PELT COMPLETED AND BEGINNING OF RETURN
TO SECOND SUMMER PELT

(November 9, 1940, to April 9, 1941)

The control (13 B) was almost completely white by November 9, 1940, and was pure white by November 20. She began to return to summer pelt before March 10, 1941, becoming brown along the mid-dorsal line by March 27, and extending it considerably by April 2. The experimental animal (14 B), meanwhile, under daily light-periods of 20 hours, put on summer pelt, out of season, beginning between November 20 and December 7, 1940, reaching almost complete summer coat by December 19 to 25. She was returned to normal daylight (9 hours, 13 minutes), suddenly, on January 6, 1941. She responded by not putting on full summer pelt on the lower part of her body and returned to brown summer coat normally about March 10.

By placing both animals under similar conditions of light, both were brown on the back and white underneath early in April.



BISSENETTE AND BAILEY. CONTROL OF CHANGES IN COAT COLOR



PLATE 4

BEGINNING OF THE REVERSED EXPERIMENTS

(April 9 1941 to December 24 1941)

Beginning with April 12 1941 weasel 14 B became the control being exposed to normal daily light conditions while the former control 13 B now became the experimental animal

Under previous experimental conditions 14 B had been brought to a fully white condition which was retained during the entire summer of 1941 and even through the winter and early spring of 1942 (see PLATE 5)

On the other hand 13 B under experimental reduction of lighting continued to increase the practically brown coat which she possessed at that time until May 16 as if by momentum At this time it was fully brown and was retained throughout the summer and even until the middle of October when it began to turn white reaching the completely white pelt on November 6 So that by the end of December both animals were fully white

PLATE 5

CONTINUATION OF THE REVERSED EXPERIMENTS

(January 13, 1942, to April 29, 1942)

The lighting of 14 B remained normal, but the white pelt which she had assumed under the previous experimental conditions, nevertheless, was retained until March 9, 1942, when, apparently, the effects of natural lighting began to show in the normal development of a brown summer pelt, which, however, remained somewhat incomplete beneath.

Under experimental conditions, 13 B, with an increase of lighting during the winter of 1941-1942, assumed a brown pelt during January and February, reaching a nearly complete condition by March 24, just as the control, 14 B, was beginning to put on a brown pelt. The lighting of 13 B was reduced in April, followed by a change to white pelt, which was beginning to show by April 29.





PLATE 6

CONTINUATION OF THE REVERSED EXPERIMENT

(May 6, 1942, to November 8, 1942)

During this period, 13 B was returned to normal lighting, beginning in March, but the influence of the previously reduced lighting continued to show in nearly complete assumption of white pelt in May and June. Toward the end of June and during July, the effects of the normal lighting, started in March, were indicated by the assumption of a brown pelt progressing until the end of July, when it stopped.

Under the now simultaneous normal lighting for both animals, both were rapidly turning white by November 8.

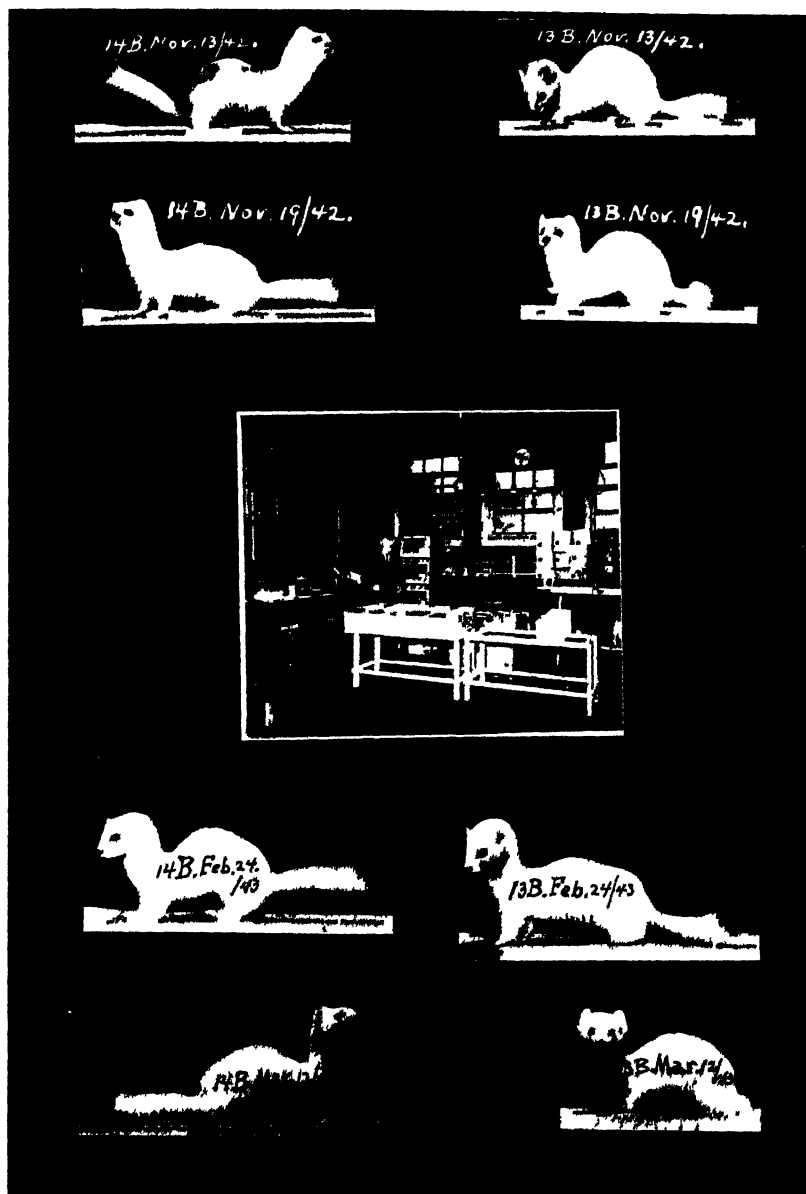
(Erratum: In the third figure from the bottom in the left-hand column, read "14 B. Oct. 29 / 42" instead of "14 B. Oct. 29 / 41.")

PLATE 7

CONCLUSION OF THE EXPERIMENTS

By November 13, very little brown or gray remained on either animal, and, by November 19, both were completely white. This coat remained all winter, starting a normal change to brown about March 12. Thus, both normal and experimental animals became synchronized to normal changes of pelt, in spite of their alternate subjection to experimental conditions.

The central photograph in this plate shows the laboratory and equipment utilized in connection with the experiments.



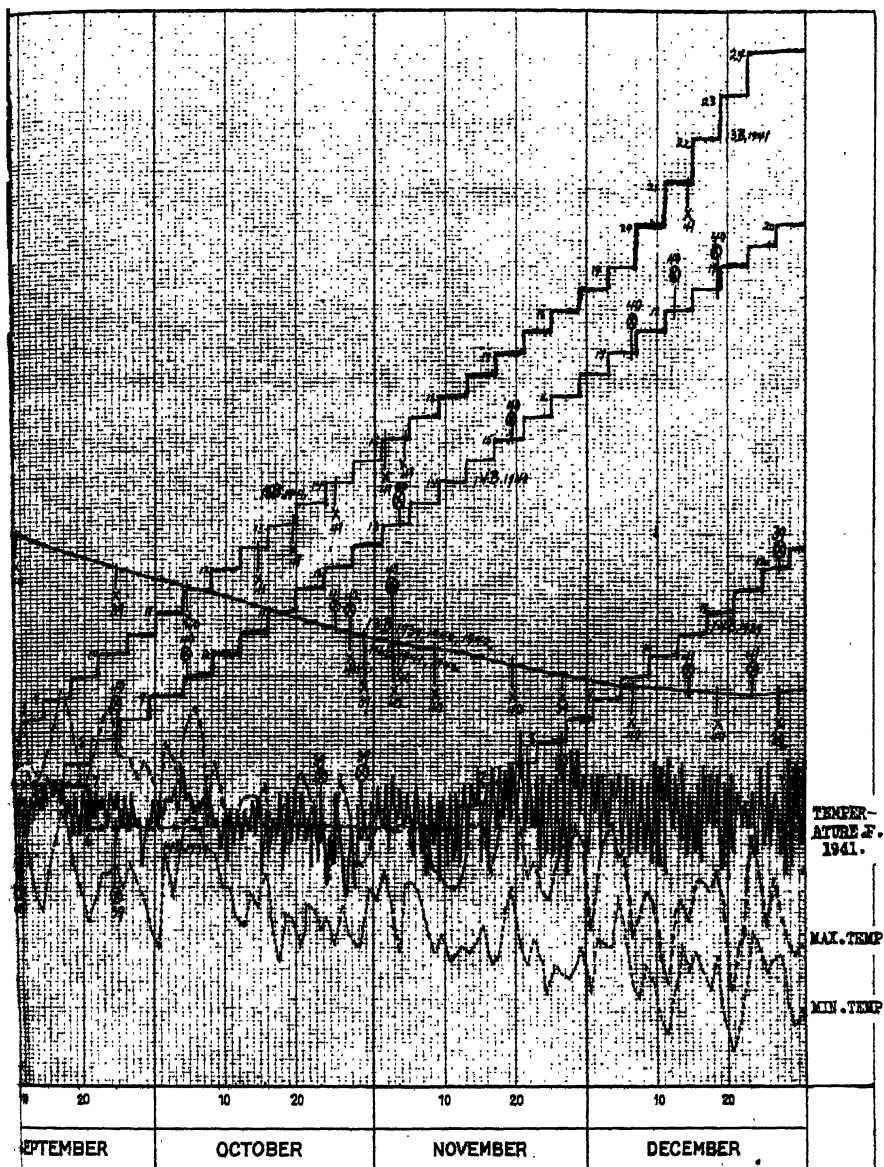
BISSONNETTE AND BAILEY: CONTROL OF CHANGES IN COAT COLOR

EXPLANATION OF GRAPH (FIGURE 1)

This graph shows (1) the number of hours of daily light to which the experimental and control animals were exposed from 1939 to 1942; (2) the temperature maintained in the experimental room during that period; and (3) the maximum and minimum daily outdoor temperatures during the course of the experiment.

KEY

- Hours of daily light—Normal: represented by the continuous smooth line
Experimental: represented by stepped line
- Temperatures—In experimental room: heavy zigzag line
Maximum outdoors: heavy broken line
Minimum outdoors: light dotted line
- Animals—14 B: Experimental during first period, 1939-1940
Control on normal days during second period, 1941-1942
13 B: Control during first period, 1939-1940
Experimental during second period, 1941-1942



THE DISTRIBUTION OF THE SALAMANDERS OF THE GENUS *PLETHODON* IN EASTERN UNITED STATES AND CANADA*

By
ARNOLD B. GROBMAN†

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* Awarded an A. Cressy Morrison Prize in Natural Science in 1943 by The New York Academy of Sciences. Publication made possible through a grant from the income of the Ralph Winfred Tower Memorial Fund.

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INTRODUCTION

Less than two decades have passed since the appearance of Dr. Dunn's (1926) excellent monograph on "The Salamanders of the Family Plethodontidae." Its inclusion of locality spot maps, showing the distribution of each form considered, and a thorough discussion of their relationships, would indicate a certain degree of temerity on the part of the present writer in presenting an account of the distribution of *Plethodon*, one of the genera of this family. There is justification for this presentation, however. Much additional material has accumulated in museum collections since 1926. In this paper, for example, there are sixteen forms recognized in the eastern Plethodons where Dunn had but eight. One entire species group (Welleri), containing three species (*richmondi*, *nettingi*, and *welleri*), was not known when Dunn's book was published. Specimens have become available from regions that were comparatively inaccessible only a few years ago due, largely, to the expansion of the public highway system. Further, an attempt is made here to elucidate the facts of distribution by the consideration of applicable information from the recent literature of the cognate fields of glacial geology, physiography, phytogeography, genetics, and experimental embryology. Finally, it should be pointed out that much new information about the genus under consideration has been made available since 1926 by Bailey, Bishop, Brimley, Dunn, Greene, Heinze, King, Mittleman, Netting, Walker, and others.

This report is designed to be a study of geographic distribution. Those who are not familiar with the salamanders under discussion may read it with most profit if a copy of Dunn's book is at hand. It is considered beyond the scope of the present work to include morphological descriptions and an analysis of systematic characters. These are to be found in Dunn's book. However, where new forms are described and where new groupings are proposed, a consideration of their systematic characters has been incorporated.

The original plan of this study was to survey the distribution of all the eastern forms of the family Plethodontidae, and this still remains the over-all plan. Because of the uncertainty connected with existing conditions, however, it was thought best to present, at this time, such material as has been compiled. Other sections, on the remaining genera, may be looked for at varying intervals in the future. A treatment of *Desmognathus* and *Leurognathus* is now in preparation.

The present paper deals with the genus *Plethodon* in eastern United States and Canada. Certain western species of *Plethodon* are not con-

sidered to be within the scope of this report. These, listed in the chronological order of their descriptions, are: *P. vehiculum* (Cooper), *P. vandykei* Van Denburgh, *P. elongatus* Van Denburgh, *P. dunni* Bishop, *P. idahoensis* Slater and Slipp, and *P. hardii* Taylor.

The eastern members of the genus may be divided into two distinct groups—one including the larger *Plethodons* with fewer costal grooves and the other, the smaller *Plethodons* with more costal grooves. These two groups differ sufficiently from each other to be considered subgenera or even distinct genera. Allocations of this nature cannot be made, however, until the western members of *Plethodon* have been brought into the scheme; here the two groups will be referred to as the Large *Plethodons* and the Small *Plethodons*. The Large *Plethodons* contain two species groups, Metcalfi and Glutinosus, and the Small *Plethodons*, likewise, are comprised of two species groups, Cinereus and Welleri. The included forms may be listed as follows:

Plethodon Tschudi

Large *Plethodons*

Glutinosus Group

Plethodon glutinosus glutinosus (Green)

Plethodon glutinosus albagula n. subsp.

Plethodon ouachitae Dunn and Heinze

Plethodon wehrlei Fowler and Dunn

Plethodon yonahlossee Dunn

Metcalfi Group

Plethodon metcalfi Brimley

Plethodon clemsonae Brimley

Plethodon jordani Blatchley

Plethodon shermani Stejneger

Small *Plethodons*

Cinereus Group

Plethodon cinereus cinereus (Green)

Plethodon cinereus angusticlavius n. subsp.

Plethodon cinereus serratus n. subsp.

Plethodon dorsalis Baird

Welleri Group

Plethodon richmondi Netting and Mittleman

Plethodon nettingi Green

Plethodon welleri Walker

DISTRIBUTIONAL CONCEPTS IN THE GENUS PLETHODON

Syngeographic Patterns

The distributional patterns of the forms of the Large Plethodons are nearly congruent with those of the Small Plethodons (FIGURES 1 and 2). In such pairs of duplicate geographic patterns the forms occurring in any one region may be termed "geographical equivalents." The term is used in a sense analogous to "ecological equivalents" of Hesse, Allee, and Schmidt (1937: 79), which term refers to either closely allied or taxonomically unrelated animals that inhabit the same ecological niche. "Geographical equivalents," then, may be defined as forms, either closely or remotely related, that inhabit the same general area. Either of two (or more) groups, each containing forms having geographical equivalence in the other group, is considered a syngeograph and, therefore, the distribution of one is syngeographic with that of the other. Syngeographs may be distinguished at any level above that of a single form, and the term is not considered synonymous with any taxonomic group.

The genus *Plethodon* in eastern United States is a syngeographical system that is comprised of a pair of syngeographs, the Large Plethodons and the Small Plethodons. The geographical equivalence existing between the forms of the syngeographs may be best understood if reference is made to TABLE 1 and the accompanying maps (FIGURES 1 and 2).

The table requires additional remarks. Although *angusticlavius* and *serratus* are regarded as geographical equivalents of *ouachitae*, they are probably phylogenetic equivalents of *albagula*. The three, *albagula*, *angusticlavius*, and *serratus*, may be considered southwestern terminal races of widespread plastic parent species.

P. c. cinereus and *glutinosus* may be considered a pair of geographical equivalents in which one member has its range displaced in a north-south direction. Although *glutinosus* has a more southern distribution than *cinereus*, the eastern and western boundaries of the ranges of the two species are virtually identical. Both extend as far west as the prairie-forest boundary; both avoid the Mississippi Alluvial Plain; and, for the most part, both reach the Atlantic Coast.

Although *wehrlei* is listed as a geographical equivalent of *richmondi*, it has not become established in the section of the Allegheny Plateaus west of the Ohio River (with two exceptions; see under that form),

TABLE 1

A TABLE OF THE GEOGRAPHICAL EQUIVALENTS AMONG THE EASTERN MEMBERS OF THE GENUS *PLETHODON*

Distributional Pattern	Large Plethodons	Small Plethodons
Forested area of the eastern U. S., excluding the Mississippi Alluvial Plain	<i>P. g. glutinosus</i>	<i>P. c. cinereus</i>
Ouachita-Ozark Uplands	<i>P. ouachitae</i>	<i>P. c. angusticlavius</i> , <i>P. c. serratus</i>
Interior Low Plateaus	Unspotted <i>P. g. glutinosus</i> ¹	<i>P. dorsalis</i>
Allegheny Plateaus	<i>P. wehrlei</i>	<i>P. richmondi</i> <i>P. nettingi</i>
Restricted areas of the Blue Ridge	<i>P. clemsonae</i> , <i>P. jordani</i> , <i>P. metcalfi</i> , <i>P. shermani</i> , <i>P. yonahlossee</i>	<i>P. welleri</i>
Edwards Escarpment	<i>P. g. albagula</i>	

¹ Approximate range indicated by a dashed line on FIGURE 1.

while *richmondi* is to be found widely distributed on both sides of the Ohio River.

There is no geographical equivalent for *nettingi* known among the Large Plethodons. Since *nettingi* is probably a localized derivative of *richmondi*, and since its range is contained within that of *richmondi*, the best allocation may be to consider *wehrlei* a geographical equivalent of *richmondi* plus *nettingi*.

In the table, *ouachitae* is listed as a geographical equivalent of *angusticlavius* and *serratus*. An alternative consideration would be to regard the Ouachita Province forms, *ouachitae* and *serratus*, as being geographical equivalents of each other, and *angusticlavius* as lacking a known southern Ozark Province geographical equivalent among the Large Plethodons.

It will be noticed that among the series, pairs of geographical equivalents will vary from each other with regard to the relative systematic position of their forms. For example, *glutinosus* and *cinereus* are widespread parent species; *ouachitae* is a distinct species while *serratus* is a race of *cinereus*; and *dorsalis* is a distinct species, whereas its geographical equivalent apparently has not yet reached a point in its history of differentiation where it may yet be regarded, taxonomically, as a subspecies.

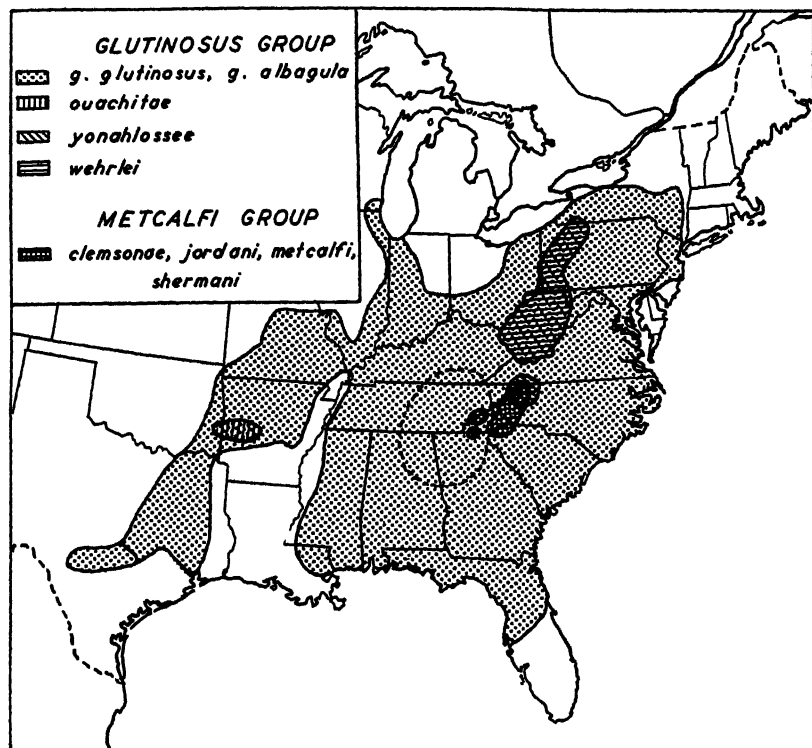


FIGURE 1. Map showing the approximate distribution of the forms of the Large Plethodons syngraph. The broken line suggests the area inhabited by the unspotted form of *P. g. glutinosus* (q. v.).

Glacial Correlations

In a study of widespread species in the eastern United States and Canada, it is usually of interest to take notice of any distributional patterns that might indicate a response to past glaciation. Bishop and Schmidt (1931: 134), considering the subspecies of *Chrysemys picta*, wrote, "It is not impossible that the two forms have differentiated while isolated during the last advance of glaciation, and that their intergradation is due to intermingling and hybridization in their meeting ground on their subsequent reoccupation of glaciated territory." Of the two subspecies of *Sistrurus catenatus*, Gloyd (1940: 77) wrote, "Since the region in which these forms intergrade coincides approximately with the most southern advance of the glaciers, and since the range of *catenatus* lies entirely within the glaciated area, it is likely that the north-

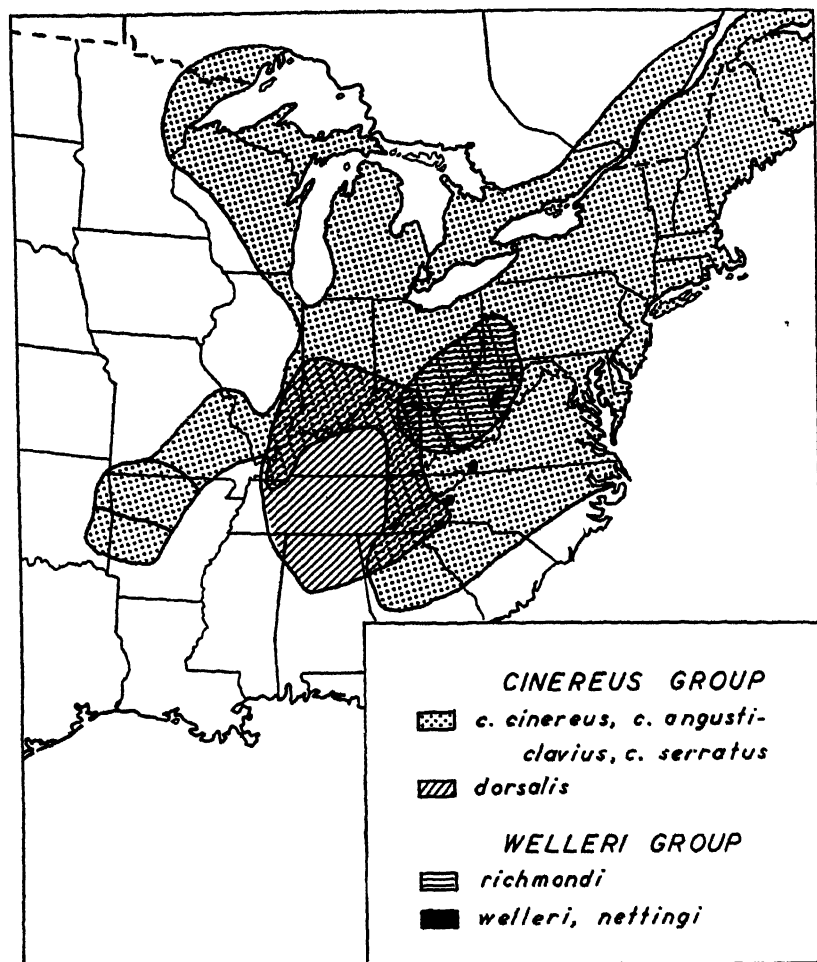


FIGURE 2. Map summarizing the approximate distributions of the forms of the Small Plethodons syngnathus.

eastward spread and differentiation of the form followed close upon the retreat of the ice."

In some herpetological literature the close correlation of the edge of a range with a glacial boundary is mentioned but not given any interpretation. Thus, Conant (1938: 14-15, 24, 47, 98, 113, 117, 130) describes some forms as restricted, or nearly so, to glaciated areas in Ohio, while others were found only in unglaciated country. It has been pointed out (Grobman, 1941a: 12) that a large portion of the southern border of the

range of *Opheodrys vernalis* coincides with the line marking the southernmost extension of the Pleistocene glaciation.

Certain herpetological papers are of interest in this connection, because they contain distribution maps suggesting range correlations with glacial boundaries, although they make no mention of these correlations. Among these may be mentioned the maps depicting the range of *Eumeces laticeps* (Taylor, 1935: 221), *Sceloporus undulatus* (Smith, 1938: 9), *Crotalus h. horridus* (Gloyd, 1940: 175), *Elaphe vulpina* (Conant, 1940: 6), *Lampropeltis getulus nigra* (Schmidt and Davis, 1941: 181), *Agkistrodon mokeson* (Gloyd and Conant, 1943: 153), *Ambystoma opacum* (Bishop, 1943: 148), *Pseudotriton r. ruber* (*ibid.*, p. 390), *Eurycea lucifuga* (*ibid.*, p. 432), and *Eurycea longicauda* (*ibid.*, p. 422).

The greater number of range maps in the above reports, however, are of reptiles and amphibians that are seemingly unaffected in their distributions by glacial boundaries. Likewise, the maps of Hamilton (1943) and Kinsey (1930) suggest, respectively, that the mammals of the eastern United States and the gall wasps of the genus *Cynips* are entirely independent of former glacier borders.

Nevertheless, as indicated above, parts of the ranges of many forms are nearly coincident with glacial limits. Two patterns, and separate corresponding phenomena, may be recognized among these ranges. They may be designated as Ecological and Temporal Glacial Patterns.

ECOLOGICAL GLACIAL PATTERN.—Under this heading may be listed ranges that are determined (primarily or secondarily) by the ecological differences that exist between glaciated and unglaciated terrains. Such ranges would be expected to terminate quite exactly along the terminal moraine of the total Pleistocene glaciations. Thus, the distribution of a form, to be considered as an example of an ecological glacial pattern, would have to lie totally within glaciated country or entirely within unglaciated terrain. In the eastern United States, this would require a correlation with the boundary marking the southernmost extension of the Pleistocene glaciation. Forms in unglaciated areas would be expected to invade glaciated country in large river valleys in which the glacial deposits had been eroded away, the river valleys providing terrain ecologically similar to unglaciated territory. A well-marked example of this is the Hudson River Valley, which is entirely within glaciated country, yet supports a fauna containing elements, *Sceloporus undulatus fasciatus* and *Pseudotriton r. ruber*, whose ranges, otherwise, are almost completely south of the terminal moraine.

The distributions of the following forms are thought to represent ecological glacial patterns: *Sceloporus undulatus fasciatus*, *Pseudo-*

triton r. ruber, *Eurycea lucifuga*, *Opheodrys aestivus* (all from unglaciated regions), and *Opheodrys vernalis* (in part; from glaciated country).

TEMPORAL GLACIAL PATTERN.—This pattern is less regular than the ecological one and is that of an animal that ranged up to the vicinity of the ice front during the glacial period and has subsequently, following the retreat of the glaciers, been able to migrate only a relatively short distance northward. Hence, the northern edge of its range would correspond only roughly with a glacial boundary. The glacial boundary to be considered would not be the southern extent of Pleistocene glaciation but rather the edge of the most recent extensive substage of the Wisconsin drift. It would be expected that the northern edge of the range of a form showing a temporal glacial pattern would be north of, and somewhat parallel to, the southern limit of the most recent glacial substage. The distance between these parallel lines would, in part at least, depend upon the vagility of the species under consideration.

The ranges of the following forms may be considered examples of temporal glacial patterns: *Plethodon g. glutinosus*, *Eumeces laticeps*, *Crotalus h. horridus*, *Agkistrodon m. mokeson*, and *Lampropeltis getulus niger*.

At least two salamanders, *Ambystoma opacum* and *Eurycea longicauda*, show the characteristics of both the ecological and temporal glacial patterns. They are found varying distances north of glacial boundaries and have a deep penetration into glaciated country in the Hudson River Valley.

Terminal Racialion

It is of interest to point out the occurrence of terminal subspecies in the southwestern extensions of the ranges of both the *Glutinosus* Group and the *Cinereus* Group. At present, these terminal forms (*P. glutinosus albagula*, *P. cinereus serratus*, and *P. c. angusticlavius*) are, it seems from available distributional data, connected with the parent form through a comparatively narrow isthmus of occupied territory. The relatively rapid fanning out of the salamanders into suitable ecological regions as they reached the terminal areas may be, in a large part, responsible for the inability of the parent forms to swamp out the differentiation that was being aided by the partial isolation. It seems that here we are dealing with cases in which racialion has accompanied, or shortly followed, the invasion of new territory.

MATERIALS, METHODS, AND ACKNOWLEDGMENTS

The distributional data for this study were largely gathered during the summer of 1942. At that time I visited the herpetological collections of the Museum of Comparative Zoölogy, Cambridge, Massachusetts; American Museum of Natural History, New York City; Academy of Natural Sciences, Philadelphia, Pennsylvania; United States National Museum, Washington, D. C.; Carnegie Museum, Pittsburgh, Pennsylvania; University of Michigan Museum of Zoölogy, Ann Arbor; and Cincinnati Society of Natural History, Cincinnati, Ohio. At these institutions, records of the eastern plethodonts were obtained from the museum catalogues and critical material examined or set aside for later shipment to Rochester. In addition, on this trip, and subsequent to it, there has been available material and/or information about the following collections: Philadelphia Zoölogical Society (at present with Roger Conant); J. T. Sackett; Sherman C. Bishop; University of Rochester; University of Arkansas Museum; Patuxent (Maryland) Game Refuge; Field Museum of Natural History; Baker-Hunt Foundation Museum; New York State Museum; and Smoky Mountains National Park. The abbreviations used in reference to collections follow:

- AMNH, American Museum of Natural History
- ANSP, Academy of Natural Sciences, Philadelphia
- BHFM, Baker-Hunt Foundation Museum
- CM, Carnegie Museum
- CSNH, Cincinnati Society of Natural History
- FMNH, Field Museum of Natural History
- MCZ, Museum of Comparative Zoölogy
- PZS, Philadelphia Zoölogical Society
- SCB, Collection of Sherman C. Bishop
- UAM, University of Arkansas Museum
- UMMZ, University of Michigan Museum of Zoölogy
- UR, University of Rochester
- USNM, United States National Museum

Deviations from the nomenclature of the 1943 edition of the Stejneger and Barbour "Check List of North American Amphibians and Reptiles" are discussed where they first appear. In the use of names for species groups, the name selected has usually been that of the oldest or best known species of the group in question. These have been written with an initial capital letter and are not italicized. Likewise, the names of the two major divisions of the genus are written with initial capital let-

ters and are not italicized. Only generic, specific, and subspecific names are in italics.

The terms "allopatric" and "sympatric," as proposed by Mayr (1942), are used here despite the objection of Hubbs (1943: 174) that they are etymological hybrid terms and should be replaced by "ali-patric" and "compatric." The base "patria" is to be found in both Latin and Greek, so it would seem that either series of prefixes might be used correctly.

When descriptive statistics are used the errors are standard errors.

Accompanying the textual descriptions of the distributions of the separate groups and forms (which follow the general pattern: first, discussion of questionable records; and, second, interpretation of the distribution) are spot locality maps. It has been said that spot maps show the distribution of collectors. If this is the only information these spot maps convey, the indication is that collectors of plethodont salamanders have been pretty widely distributed throughout the eastern United States.

The base map used for most of the illustrations of distribution is an outline map of the United States from the July 1, 1940, edition of the Bureau of Agricultural Economics of the Department of Agriculture. These have been procured with blue county boundaries, so that these boundaries would not appear in reproduction, yet would be an aid to accurate plotting. The maps were reoriented in preparing them for publication.

The localities plotted on the smaller scale maps are accurate to county. This introduces slight inaccuracies in those states with large counties, for the spot has been placed in the approximate geographic center of the county regardless of the exact locality of capture. The spots on the map of the Metcalfe Group are believed to be accurate to within two or three miles of the point of collection. It has been the policy to use solid symbols for localities from which museum specimens have been seen and to use hollow symbols for additional localities that have been reported in the literature. The literature, from which locality records have been accepted, and from which no citations have been made, is not included in the list of Literature Cited. Most of the questionable literature records have not been omitted from the maps without a discussion in the accompanying text, except in those instances in which they had been considered by Dunn (1926) or Bailey (1937).

Certain areas have been less well represented than was possible with the available material. It had been hoped that Dr. Charles Walker's forthcoming "Amphibia of Ohio" and the "Herpetology of Illinois,"

being prepared under Dr. Howard K. Gloyd's guidance, would both be available before the completion of this report and, therefore, only a cursory study was made of the plethodont salamanders of Ohio and Illinois.

I am under obligation to many persons who have given me kind advice and help in numerous ways. For permitting me to examine the museum collections under their custody, in offering many courtesies associated with these examinations, in supplying information, and in subsequently loaning me critical material, I am deeply indebted to Mr. Charles M. Bogert, Dr. Doris M. Cochran, Dr. E. R. Dunn, Mr. Ralph Dury, Mrs. Helen T. Gaige, Dr. Norman Hartweg, Mr. Arthur Loveridge, Mr. M. Graham Netting, and Dr. James A. Oliver. Mr. Roger Conant has graciously made available material that is to be deposited with the Philadelphia Zoölogical Society, and Mr. William H. Stickel, in a like manner, has shown me the salamanders in the Patuxent Game Refuge Collection which were gathered largely through the efforts of Mr. Francis M. Uhler. Mr. J. T. Sackett has permitted me to include data on salamanders he had collected in Rhode Island. I have borrowed specimens from other collections not visited during the summer of 1942 and thank Dr. S. C. Dellinger, Mr. Karl P. Schmidt, and Mr. Arthur Stupka for lending this material to me.

Mr. Ray Maas has been helpful in photographing the maps, figures, and charts.

Several interested persons have criticized parts of the manuscript and have been extremely helpful with their suggestions. Discussion and correspondence with them have been the sources of many stimulating comments. Especially to be mentioned in this regard are: Dr. Joseph R. Bailey, Dr. Donald R. Charles, Mr. Coleman J. Goin, Miss Hulda Gross, and Dr. Hobart M. Smith. This report has greatly benefited from some of the discussions with Dr. Norman Hartweg and from his criticisms of a major part of the manuscript. Mr. M. Graham Netting has generously supplied me with much novel information about the genus and to him should go the major share of the credit for the section on the Metcalfi Group. He has also read most of the manuscript. Finally, it is a pleasure to acknowledge my debt to Dr. Sherman C. Bishop. He has put at my disposal his very excellent collection of United States salamanders, his substantial herpetological library, and his very complete species-geographic file of United States salamander literature. He has guided this research most sympathetically, has been continuously available for the discussion of problems as they arose, and has read the entire manuscript.

SPECIFIC DISTRIBUTIONAL ACCOUNTS

The Glutinosus Group

In the present paper the following five forms are recognized in the Glutinosus Group:

Plethodon glutinosus glutinosus (Green)

Plethodon glutinosus albagula n. subsp.

Plethodon yonahlossee Dunn

Plethodon wehrlei Fowler and Dunn

Plethodon ouachitae Dunn and Heinze

This group is most closely related to the Metcalfi Group of *Plethodon* and the relationship is discussed under that heading.

The relationship, both geographically and morphologically, between *yonahlossee* and *wehrlei*, is very close. Separating the ranges of the two forms is the narrow Valley and Ridge Province where it cuts through northwestern Virginia. This region should be carefully collected for members of the *yonahlossee-wehrlei* cline. The cline exists in size, disposition of white pigment, and disposition of red pigment. From *yonahlossee* through southern *wehrlei* to northern *wehrlei* there is a reduction in size. Dunn (1926: 132) gives 165 mm. as the total length of the largest individual of *yonahlossee* he has measured. He (*ibid.*, p. 135) records his largest *wehrlei*, from Parkersburg, West Virginia, at 152 mm., and Bishop (1941a: 235) gives 135 mm. as the largest total length measurement for *wehrlei* from the Allegany State Park district, New York. Through the same chain of forms the white pigment, forming the lateral bands of *yonahlossee* and southern *wehrlei*, is more scattered over the dorsal surface in northern *wehrlei*. This is probably, however, a correlative of environmental temperature as suggested under the *glutinosus* account. The amount of red pigment on the dorsum is reduced from south to north. In *yonahlossee* there are paired dorsal red spots in the young which Dunn (*ibid.*, p. 131) believes are the forerunners of the chestnut dorsal band of the adult. In southern *wehrlei* there are red spots in the young and in occasional adults. In northern *wehrlei* red is not found in either the young or adults (*vide* discussion under *wehrlei*). The actual present relationship between *wehrlei* and *yonahlossee* may well be subspecific, and positive evidence concerning such a disposition should be looked for in material from the above-mentioned region.

The Glutinosus Group may be described as being comprised of a widespread variable species (*glutinosus*) with one recognized geographic

race (*albagula*) and three distributionally restricted species (*ouachitae*, *yonahlossee*, and *wehrlei*) that have a sympatrical relationship with the widespread form (FIGURES 3 and 4). The three restricted species are more closely related to each other than any of them is to *glutinosus*. About *P. ouachitae*, Dunn and Heinze (1933: 122) wrote:

"This species is closely allied to *Plethodon wehrlei* of the Appalachian Plateau in New York, Pennsylvania and West Virginia, and to *Plethodon yonahlossee* of the Southern Blue Ridge in Virginia and North Carolina. It is more like *yonahlossee* in coloration, and more like *wehrlei* in dentition and proportions. It is extremely interesting to find another Appalachian type in this isolated mountain region, and bespeaks a quite respectable age for the *yonahlossee-wehrlei-ouachitae* group."

More recent information (Bishop, 1943: 271, 284, and 289), however, seems to indicate that in dentition, at least as far as the number of vomerine teeth is concerned, *ouachitae* and *yonahlossee* are at least as close to each other as *ouachitae* is to *wehrlei*. Bishop (*ibid.*) gives the following range of counts for the three forms: *ouachitae*, 7-13; *yonahlossee*, 10-14; and *wehrlei*, 6-9.

The present distribution of the members of the *Glutinosus* Group may be accounted for in several ways. Two of the more interesting and extreme explanations are given here. It has been suggested by Dunn and Heinze (*op. cit.*) and by Dunn (1926: 23, 133, 135) that the *yonahlossee-ouachitae-wehrlei* subgroup is older and more primitive than the species *glutinosus*. An expansion of this thesis, and the one with which the nomenclature of the present paper is in accord, would suggest that the red-marked forms (*yonahlossee*, *ouachitae*, and *wehrlei*) were early migrants with a center of dispersal in the southern Appalachians. *P. wehrlei* would be a "somewhat degenerate northern form" (*ibid.*, p. 133) of *yonahlossee*, and *ouachitae* would be a relict species of the Ouachitas dating back to the time when *yonahlossee*, "the most primitive . . . of the whole genus *Plethodon*" (*ibid.*), was widespread over the eastern United States. At some subsequent time, the forerunner of *glutinosus* was differentiating from the existing *yonahlossee* stock. During or following the restriction of the members of the *yonahlossee-ouachitae-wehrlei* subgroup to their present-day ranges, *glutinosus* spread peripherally, presumably from the southern Appalachians. The picture today in the *Glutinosus* Group, then, is one of a young, widely distributed, and morphologically variable species (*glutinosus*) with several incipient, or undescribed, geographic races (*vide* under *glutinosus*) and a

series of old, static, relict species (*wehrlei*, *yonahlossee*, and *ouachitae*) with reduced and restricted ranges.

This picture forces to the fore the question concerning the relationship between *ouachitae* and *yonahlossee*. These forms are very similar and in the small series available to me here I could detect no differences (aside from those that might be considered ontogenetic in nature) other than that the venter of *yonahlossee* seemed to be somewhat lighter than that of *ouachitae*. Whether this difference is constant and genetic I do not know. It may be that, genetically as well as phenotypically, these assemblages are very similar and represent the parent populations with but few changes. This interpretation would indicate a discontinuous distribution for *yonahlossee* and would require placing *ouachitae* in the synonymy of *yonahlossee*. Experimental and morphological studies would be necessary to determine whether or not these populations have diverged sufficiently to be considered separate species, subspecies, or undifferentiated populations of the same species.

The other explanation of the present distribution of the *Glutinosus* Group to be mentioned in this paper has *glutinosus* a widespread form with, for example, monogenic mutant populations that had arisen and hung on in favorable upland areas, as in the southern Appalachians (*yonahlossee*) and in the Ouachitas (*ouachitae*). (Vide Mittleman's suggestion under *ouachitae*.) This explanation does not account for the obviously close relationship between *wehrlei* and *yonahlossee* and the distinctness between the former and *glutinosus*.

In the present paper, the existing nomenclature is used but it is indicated that the taxonomic problem needs additional work.

Plethodon glutinosus glutinosus (Green)

Aside from the records designated as erroneous by Dunn (1926: 143), there is a specimen (CM 10553) allegedly collected on the east shore of Lake Abitibi in Quebec. Besides the fact that this locality is 400 miles north of the next nearest record, the probability of the data being erroneous for this specimen is enhanced because it is an Atkinson-collected specimen. As pointed out previously for an Atkinson Texas record for *Opheodrys v. vernalis* (Grobman, 1941a: 15), the locality data were almost certainly in error. Another Atkinson Texas specimen may also have erroneous locality data. About it, Malnate (1939: 363) wrote, "*Rhadinaea flavilata* is decidedly a lowland form. Locality records show it to be confined to a narrow coastal strip, and no specimen has been collected at an altitude in excess of 120 feet, with the single exception of the one Texas specimen, collected at 620 feet." The weight of

such cumulative evidence would seem to indicate that the locality data accompanying Atkinson specimens should be accepted only with extreme caution.

The western edge of the range of *P. g. glutinosus* approximates the line marking the western extent of the eastern forest flora (FIGURE 3).

This correlation might have been anticipated, for Bishop (1941a: 228) wrote, "The slimy salamander is commonly found beneath logs and stones in woods, in the crevices of shale banks and along the sides of gullies and ravines."

It further seems that either *glutinosus* does not occur in the river-bottom forest area or that collectors of *glutinosus* have not been active there. It is more likely that the former is the case and that *glutinosus* habitats are largely absent from the lower Mississippi Valley, perhaps due to the recurrent floods.

The hypothesis advanced here is that, during Wisconsin glacial times, *glutinosus* was forced southward by the advance of the ice but remained relatively close to the drift border. As the glacier finally retreated northward, the accompanying boreal flora of the border country became replaced by an ecological succession which resulted in conditions (probably not unlike those existing today) which offered many suitable habitats for *glutinosus*. Thus *glutinosus* was slowly able to spread northward until it reached its present-day range. It may still be spreading. (It is conceivable that *glutinosus* may formerly have had a wider range and that its present distribution is the result of a reduction.)

Actually, the northern edge of the range of *glutinosus* in Indiana, Ohio, and Wisconsin, corresponds more closely with the Cary Substage (Third Substage) of the Wisconsin glaciation than it does with the boundary of the maximum extent of Wisconsin drift which is shown on the accompanying map (*vide* Thwaites, 1934: 97). The substages (Early and Late Mankato) subsequent to Cary were represented by glacial drifts that did not reach as far south as the Cary Substage, and their terminal moraines are to be found at latitudes north of Lake Erie. During the period following the retreat of the Cary ice (Forest Bed Subinterval), the climate was somewhat cooler than it is today; that of east-central Wisconsin approximating that of present-day northern Minnesota (*ibid.*, p. 83). It seems likely, then, that conditions were such that *glutinosus* was able to migrate northward after the Cary Substage and that the advance of this drift forced from the area any previous populations. Except for the lakes formed by the retreating ice (e.g., Lake Maumee in northeastern Ohio), there apparently was little that could have interrupted the northern spread of *glutinosus* subse-

quent to the Cary retreat. The climate was not hostile, trees were present (*ibid.*), and the later drifts were far to the north.

Dr. Ernst Antevs (in a letter of June 3, 1943) kindly supplied me with his estimates as to the ages of the several substages. He suggests that Cary began some 35,000 years ago and that the next younger stage began some 25,000 years ago. It would seem that *glutinosus* had at least 30,000 years in which to migrate subsequent to Cary times.

The Cary drift may possibly have extended approximately as far south as the Wisconsin terminal moraine in Pennsylvania, New York,

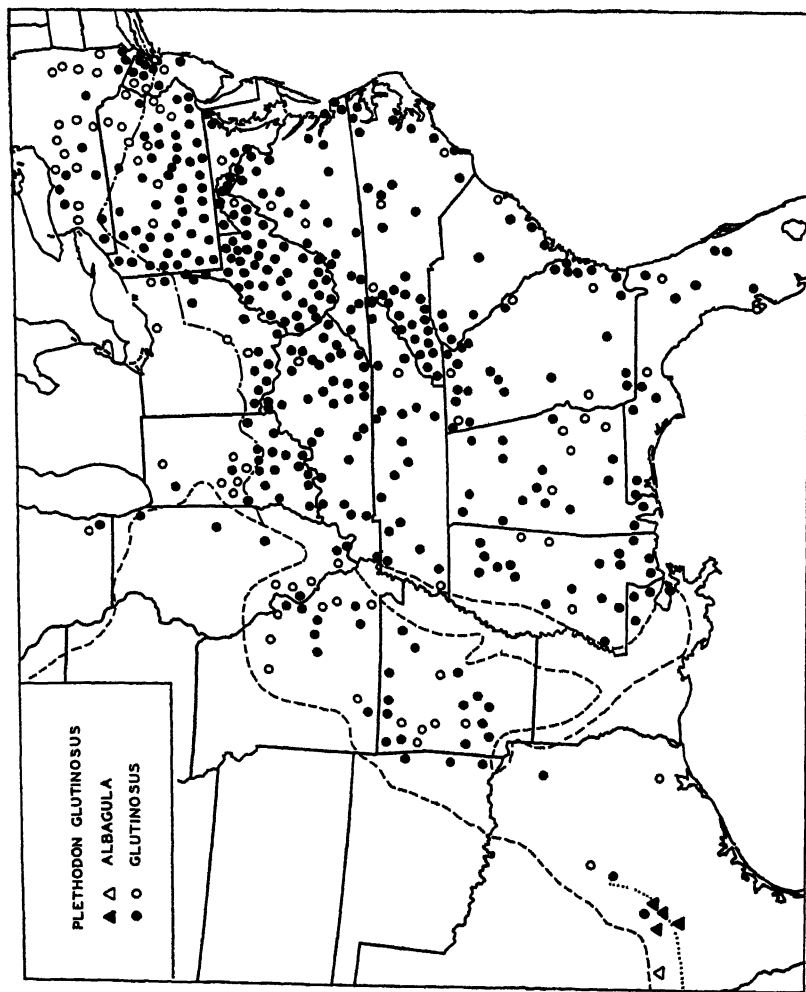


FIGURE 3

and New Jersey (*ibid.*). If this is so, then *glutinosus* would have traveled at least 150 miles beyond the terminal moraine in reaching the station northernmost from the moraine, Rotterdam Junction, Schenectady County, New York (Bishop, 1941a: 231). It would seem, therefore, that *glutinosus* migrated, on the average, one mile every 200 years (150 miles in 30,000 years). *P. c. cinereus* has, however, in the same space of time been able to traverse at least 800 miles to Cape Breton Island (Verrill, 1863: 199), thereby averaging at least one mile every 37 years. This estimate may be too high by a factor of two, for it is conceivably possible that the populations of Cape Breton Island and Gaspé Peninsula may antedate the Pleistocene glaciation and that these areas, having been free of Pleistocene ice, may have been northern dispersal centers for *cinereus*.

Besides *P. g. albagula*, which is described in this paper, there are other less readily distinguishable races of *glutinosus*. These latter races are difficult to define and it seems that a study of the pattern and coloration in living material would probably be a necessary prelude to their description.

Coleman J. Goin has told the writer that a series of *glutinosus* (CM 18797) he collected one mile south of Kingsland, Camden County, Georgia, has golden spots on the dorsum and lacked the dorsal white markings in life. This may be the conditions of *glutinosus* in the low coastal plain of southeastern Georgia and adjacent northeastern Florida.

There is, apparently, a large *glutinosus* devoid of white markings which ranges south from the Cumberland Plateau. Localities for this form are in Bibb, Chilton, Lawrence, and St. Clair Counties, Alabama; Chatooga, Dade, DeKalb, Fulton, Gilmer, and Murray Counties, Georgia; Edmonson, Harlan, Laurel, Morgan, and Whitley Counties, Kentucky; Pontotoc and Webster Counties, Mississippi; Avery, Buncombe, Cherokee, Graham, Haywood, Macon, Polk, Swain, and Transylvania Counties, North Carolina; Greenville and Pickens Counties, South Carolina; and Claiborne, Cumberland, Davidson, DeKalb, Fayette, Fentress, Rhea, and Sevier Counties, Tennessee.

Excluding certain exceptions, some of which are listed below, there seems to be an irregular cline associated with the disposition of the

FIGURE 3. Map showing the distribution of the races of *Plethodon glutinosus*. The solid symbols represent county localities from which museum specimens have been examined; the hollow symbols, from which literature reports have been included. The western extent of the eastern forest flora is marked by a broken line. Also indicated by a broken line is the area in the lower Mississippi River Valley that is vegetationally dominated by a river-bottom forest (cypress-tupelo-red gum) (*vide* Shants and Zon, 1924: 5). The dot-dashed line in the north represents the southern extent of the most recent (Wisconsin) Pleistocene ice sheet, as mapped by Antevs (1929: 640, 642, 644-5). The dotted line in Texas approximates the position of the Balcones Escarpment. The apparent absence of *P. g. glutinosus* from the Delaware-Maryland-Virginia peninsula and southern New Jersey is noteworthy.

white pigment. (Coastal plain material is usually but sparsely marked. A proportion of the specimens is unmarked. This has previously been pointed out for individuals from the Charleston, South Carolina, region by Cope (1889: 142), Schmidt (1924: 68), and Dunn (1926: 139).) North of the coastal plain, the cline is from the mountains (around the Georgia, Tennessee, and North Carolina border area) northward. The southern members of the cline have the white pigment absent from the dorsum and concentrated on the sides to form lateral bands. In material from more northern stations, the pigment is more diffuse with spots appearing on the dorsum and with the lateral bands being less distinct. Farther north, e.g., New York state, the fewer spots are randomly distributed over the sides and dorsum; no lateral bands are present. One of several explanations involves some sort of temperature gradient. Twitty (1936: 239-302) and Twitty and Bodenstein (1939: 357-398), working with western *Triturus*, describe the larvae of *torosus* as possessing dorsal bands and an unpigmented lateral area, and of *rivularis* as having the pigment uniformly scattered. Experiments, keeping neural crest cells *in vitro*, suggested that a time factor is important and that the difference between the *torosus* and *rivularis* larval patterns is merely a function of the slower rate of development of the latter species which would permit a farther migration of its pigment-bearing cells (Du Shane, 1943: 117, is not in accord with this interpretation). Something akin to this may be the basis for the varying patterns of *glutinosus*. Hubbs (1926: 57-81) has similarly explained clines in races of fishes in which the number of scales and spines increases from south to north and from warm waters to colder waters. The developmental rate is undoubtedly strongly influenced by the temperature and the developmental rate may control the pattern of *glutinosus* as it seems to in the western *Triturus*. Thus, an irregular temperature gradient might be intimately correlated with an irregular pattern cline.

Exceptions to this cline, in the form of individuals or populations with strong white lateral bands, are known from several localities. Among these are: Imboden, Lawrence County, Arkansas (AMNH 35887-91); Ashland, Franklin County, Georgia (AMNH 34640); southern Illinois (AMNH 5834-5); Bethel Ridge, Green County, New York (photograph in M. Graham Netting's possession of a specimen collected and photographed by George G. Wilmott); near Williams Mill Pond, Gates County, North Carolina (USNM 84206-7); near Camp Davis, Onslow County, North Carolina (PZS 2684); Big Sandy, Benton County, Tennessee (USNM 45957); and Bedford County, Virginia (USNM 36710-1). These individuals, or populations, may have been

exposed to unusual temperatures during the critical periods of their developments, or may be of somewhat different genotype from the great mass of the population.

Plethodon glutinosus albagula NEW SUBSPECIES

Holotype.—CM 9652, adult male, collected 20 miles north of San Antonio, Bexar County, Texas, by Wesley Clanton, on February 24, 1935.

Paratypes.—CM 9651, 9653–63, topotypes; SCB, Heindrich Cave, 3 miles west of Braunfels, Comal County; CM 21946–7, 21950–9, UMMZ 64052 (5), AMNH 22650, FMNH 26251–2, 37669–79, San Marcos, Hays County; AMNH 22651–9, Posey Cave, 7 miles west of San Marcos, Hays County; FMNH 37666–8, Wimberley, Hays County; SCB, Schneider Cave, 13 miles northeast of Boerne, Kendall County; and SCB, Prassell Ranch Cave, 14 miles northeast of Boerne, Kendall County; all in Texas.

Diagnosis.—A race of *Plethodon glutinosus* in which the arrangement of the black pigment to form small compact circles has been broken down in the gular region, thereby presenting a gross appearance of a lighter throat than that found in the typical race of *glutinosus*.

Description of the Type Series.—In the midline area of the throat, just anterior to the gular fold, of 96 per cent (134 out of 139 specimens examined) of a sample of typical *glutinosus* selected from various parts of its range (Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, Missouri, New York, North Carolina, Ohio, Pennsylvania, South Carolina, and eastern Texas), the disposition of the black pigment is in a network which borders subequal pigmentless circular areas. In 97 per cent (63 out of 65 specimens examined) of the members of the type series of *albagula*, the rete in this gular region is broken down in such a way that the black pigment occurs in a pattern of tri-radiate spots, there not being sufficient pigment to permit the joining of the arms of adjacent pigment spots to form intermediate pigmentless circles. Such an arrangement of pigment in *albagula* may be directly derived from the condition in typical *glutinosus* by a reduction in the amount of pigment. Apparently the pigment disappears first from those areas in which it is least concentrated, i.e., where two adjacent pigmentless circles are almost in contact tangentially, and remains longest where it is most concentrated, i.e., in the tri-radiate area bounded by three contacting pigmentless circles. This reduction in the amount (or extent) of the black pigment gives the throat of *albagula* a lighter appearance than is found in typical *glutinosus* and, in preserved material, the throat

appears whiter. In some extreme cases the black pigment is almost gone.

There is, apparently, no difference in the number or disposition of the vomerine teeth between *albagula* and *glutinosus*. In 51 adult specimens of the former the average number of vomerine teeth (total of both series) is 19.3; in 38 similar individuals of *glutinosus* the comparable figure is 19.2. There may be a sexual dimorphism with regard to the number of vomerine teeth in *albagula*, however. The counts for eleven males average 16.8 ± 0.86 and the mean for an equal number of females is 20.1 ± 0.99 .

The body proportions and size of *albagula* are included within the dispersion of *glutinosus* for those characteristics.

In general, the white pigment in *albagula* is concentrated along the sides to form a broad lateral band with the dorsum almost devoid of white pigment spots. In this way, *albagula* differs from *glutinosus* of the vicinity of the type locality (Princeton, New Jersey; *vide* Stejneger and Barbour, 1943: 17), but this pattern is duplicated by *glutinosus* populations from other parts of its range (*vide* under *glutinosus*).

Remarks.—All of the habitat labels that accompany the paratypes of *albagula* mention caves. The disintegration of the throat pattern may be a correlative of the cave habitat of *albagula* and may represent a very early stage of loss of pattern through which may have passed such forms as *Typhlotriton*, *Typhlomolge*, and *Haideotriton*.

This race has been known² for some time by many present-day herpetologists. Among these have been Karl P. Schmidt, M. Graham Netting, and S. C. Bishop, each of whom was long aware of the existence of *albagula*. To each of these investigators I am indebted for their assent to my describing this race.

The name *albagula* refers to the comparatively white throat of preserved material.

Range.—*P. g. albagula* is definitely known from seven localities in Bexar, Comal, Hays, and Kendall Counties in Texas. These localities are in a limestone region (Fredericksburg and Trinity Groups of the Lower Cretaceous) in the vicinity of the Balcones Escarpment which, at this point, marks the boundary between the Edwards Plateau and the West Gulf Coastal Plain. This is a region that is fairly heavily dissected and has many canyons and caves. The flat upland surface is only moderately wooded, but "outliers of the Atlantic timber belt are . . . found in the canyons of the Edwards Plateau" (Hill, 1900: 12).

² On the September, 1939, price list of Byron C. Marshall's Ozark Biological Laboratory, the following item may be found, "*Plethodon glutinosus*, Slimy salamander, Texas, different from more northern ones being much lighter color on chin, soles of feet, etc. .30."

Strecker (1935: 32) has reported *P. glutinosus* from West Frio Canyon, Real County, Texas. This locality seems to be in a terrain that is similar to, and contiguous with, that in which the *albagula* localities lie. This may well be an *albagula* locality and is represented by a hollow triangle on the map (FIGURE 3).

One specimen (CM 6129) from near Blanco City, Blanco County, Texas, has the well-formed pigmentless circles of *glutinosus*, although its locality is similar and very close to those of paratypic *albagula*. This specimen may well be a dark, extreme individual of *albagula*, but, until the constitution of the population at this locality is known, it is tentatively referred to *glutinosus* (represented by a solid circle on the map).

Plethodon ouachitae Dunn and Heinze

Two poorly preserved specimens (USNM 100777-8), listed on my data cards as from Dora, Howard County, Arkansas, may represent an error of record. The available Rand McNally Atlas (1934) lists a Dora, Arkansas, in Crawford County, but not in Howard County. Crawford County, however, is outside of the apparent range of *ouachitae*. Between several of the validly represented localities (Rich Mountain, Polk County, Arkansas, and Page, Le Flore County, Oklahoma) is Howard, Polk County, Arkansas. This may be the locality the specimens in question represent.

The species is known from three mountain ranges of the Ouachita Mountains (FIGURE 4). Localities represented in the Rich Mountains include: Rich Mountain (USNM 92484), six miles northwest of Rich Mountain (USNM 99621), and three miles west of Acorn (MCZ 21443), all in Polk County, Arkansas; and Page (CM 7141), Le Flore County, Oklahoma; Caddo Mountains (UMMZ 82791), Caddo Gap, Montgomery County, Arkansas. Our knowledge of the occurrence of this form in the Zigzag Mountains depends upon a specimen (CSNH 2096) identified as *ouachitae* that bears the data, Hot Springs National Park.

Mittleman (in a letter of October 10, 1942) does not agree with the present disposition of *ouachitae*. He is inclined to consider it a local color variant of *glutinosus*, the situation being reminiscent of the two color phases of *P. c. cinereus*.

Plethodon wehrlei Fowler and Dunn

Plethodon wehrlei is an inhabitant of that part of the Unglaciaded Allegheny Plateau Section of the Appalachian Plateaus Province that is north of the Big Sandy River and east of the Ohio River (FIGURE 4).

No specimens are yet known from southwestern West Virginia. It

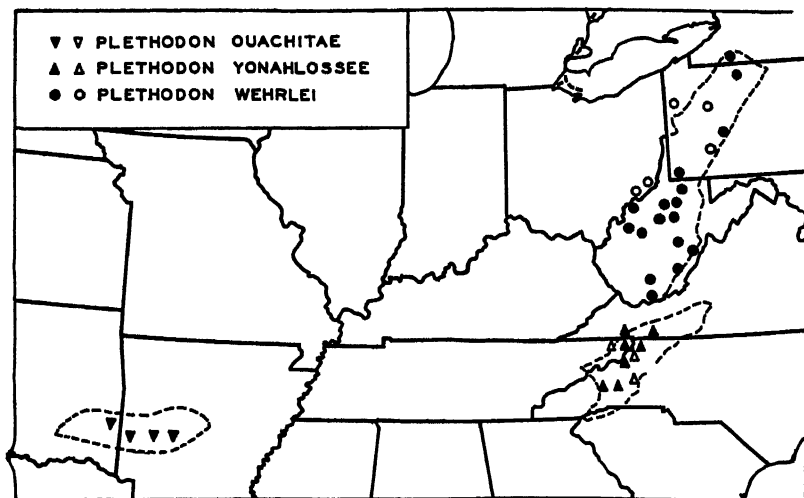


FIGURE 4. Map showing the distributions of the members of the Glutinosus Group of *Plethodon* except the races of *P. glutinosus*. Solid symbols are used to indicate county localities for museum specimens; hollow symbols, for literature records. The broken line about the *yonahlossee* stations bounds the Southern Section of the Blue Ridge Province north of the French Broad River. The broken line encircling the *ouachitae* records represents the boundary of the Ouachita Mountains as given by Fenneman (1938: plate VI). The broken line, and portions of the state boundaries involving Ohio, West Virginia, Kentucky, and Virginia, define the area of the Unglaciated Allegheny Plateau Section of the Appalachian Plateaus Province that is north of the Big Sandy River and east of the Ohio River.

may be that the New-Kanawha Rivers restrict the southwestern spread of *wehrlei*.

The records (Walker, 1933: 224) of *wehrlei* from the eastern part of Washington County, Ohio, and from Sunfish Creek, Monroe County, Ohio (represented by open circles on the map), are interesting exceptions to the suggested effectiveness of the Ohio River as a barrier to the westward spread of *wehrlei*. There is no apparent reason to believe that, at present, the Ohio River, in this region, is less effective as a barrier than it is farther north or south. *P. wehrlei* probably crossed into Ohio during preglacial, or early interglacial, times. During this period, the Ohio River was composed of two streams, one flowing northward and the other flowing southward, with the divide somewhere between Moundsville and New Martinsville, West Virginia (Fenneman, 1938: 303, 318). This area is just opposite the Monroe County, Ohio, record and it seems likely that *wehrlei* was able to migrate into Ohio from West Virginia during Pre-Wisconsin times. The desirability of a study of Ohio *wehrlei* is indicated, since these populations may have been isolated from the main stock of *wehrlei* for over 43,000 years.

The same type of north-south gradient described for *glutinosus* is also

observable in *wehrlei*. *P. wehrlei* from West Virginia, for example, has the white pigment concentrated on the sides and dorsum. Dunn (1926: 133) and others have mentioned the presence of paired red spots on the dorsum of young specimens from West Virginia, but Bishop (1941a: 238) has indicated that young northern (New York and McKean County, Pennsylvania) examples he has seen do not have red pigment showing on the dorsum. Southern *wehrlei* also appear to attain a greater size than do northern individuals. The variation within this species is now being studied at the Carnegie Museum.

Plethodon yonahlossee Dunn

This species seems to be restricted to the Southern Section of the Blue Ridge Province north of the French Broad River (FIGURE 4).

The Metcalfi Group

In the Metcalfi Group I recognize the following four forms:

- Plethodon metcalfi* Brimley
- Plethodon shermani* Stejneger
- Plethodon jordani* Blatchley
- Plethodon clemsonae* Brimley

The members of this group are most closely related to the members of the Glutinosus Group, from which they differ in a variety of characters. Adult members of the Metcalfi Group lack the white pigment patches characteristic of the Glutinosus Group adults, although certain populations of *glutinosus* (*vide* under that form) lack the patches, and examples of *metcalfi* and *shermani*, at least, occasionally possess them. The lichen-like gray markings of the Jocassee, *clemsonae*, are apparently of a different nature from the markings in question. There are usually fewer teeth in the vomerine series of individuals of the Metcalfi Group, although *wehrlei*, of the Glutinosus Group, also has a low number (6 to 9, *vide* Bishop, 1941b: 236). The average costal groove count for the members of the Metcalfi Group is about one-half groove lower than the average for the Glutinosus Group. A reddish dorsal band is known only in two members (*yonahlossee* and *ouachitae*) of the Glutinosus Group and is not present in the Metcalfi Group. Conversely, restricted red markings, on cheeks and legs, are limited to two members (*shermani* and *jordani*) of the Metcalfi Group and are not found, except rarely, in the Glutinosus Group. Members of the Glutinosus Group are, on the whole, stockier-bodied and less delicate in appearance than the members of the Metcalfi Group. Although this last characterization is difficult

to apply, and is reversed in some instances (e.g., northern *wehrlei* as compared with *clemsonae*), it is less useful as a descriptive character than a meristic one, only because of the failure of present methods to accurately measure such general proportion characters (*vide* Dunn, 1926: 46).

The two groups under consideration have been regarded by Dunn (1926: 135, 146) and Bailey (1937: 7) as closely related although recognizably different. More recently, Bishop (1941b: 18) has suggested an even closer relationship between the two groups by proposing the combination *Plethodon glutinosus shermani* (*vide* discussion under *P. shermani*).

The Metcalfe Group is quite localized in its distribution. It ranges, at altitudes between 900 and 6400 feet, through a large part of the Southern Section of the Blue Ridge Province (FIGURE 5). The distributions of the included forms are readily described in terms of mountain ranges. Apparently, *jordani* is a form of the Great Smoky Mountains; *shermani* of the Nantahalas; *clemsonae* of the Southern Blue Ridge (*sensu stricto*); and *metcalfe* of the Unakas, more northerly portions of the Southern Blue Ridge, and ranges between the Southern Blue Ridge and the Unakas.

Bailey (1937: 5) has suggested that the Little Tennessee River forms the eastern boundary of the range of *Plethodon shermani*. Both *clemsonae* and *metcalfe* are found east of this river (represented, in part, in FIGURE 5, by the broken line bounding the *shermani* records to the north and east) and such material shows no morphological approach toward *shermani*. Intergradation between *clemsonae* and *shermani* should be looked for in the region of the headwaters of the Little Tennessee; this is, presumably, the area in which the two forms meet. Material from Black Rock (just west of Rabun Gap, Rabun County, Georgia) and the ridges directly northwest (High Knob, Doubletop Mountain, Ridge Pole, Pickens Nose, etc., in Macon County, North Carolina) should be of interest in this regard. Since intergradation between *shermani* and other members of the group is not known, and since specimens from the periphery of the range are not morphologically closer to the neighboring form than are *shermani* from geographically more remote regions, it would seem desirable at present to regard *shermani* and *clemsonae* as allopatric species.

Northward, along the Little Tennessee, the river is probably a barrier to a mixture of the populations of *shermani* and *metcalfe* and, therefore, geographic intergradation between these two forms is very unlikely. Genetic exchange, then, between *metcalfe* and *shermani* could occur

only through populations of *clemsonae*. Since *shermani* and *clemsonae* are herein regarded as allopatric species, it is necessary also to consider *shermani* and *metcalfi* as allopatric species.

It would seem that *shermani* is also effectively isolated from *jordani* by the Little Tennessee River, and there is no more reason for inferring a subspecific relationship between these two forms than between *shermani* and *metcalfi*.

As the ranges of *jordani* and *shermani* are delineated (FIGURE 5), there is an area between the western halves of the two ranges from which I have seen no members of the Metcalfi Group. It may be that another vicarious form of the Metcalfi Group will be found in the Yellow Creek Mountain, Cheoah Mountains, and Snowbird Mountains (Graham, Swain, and Cherokee Counties of North Carolina) of this area.

It seems likely that *jordani* and *metcalfi* meet without intergrading, as is suggested under the *metcalfi* account.

Netting (in letters of February 13, 1943, and April 14, 1943) has suggested that *clemsonae* and *metcalfi* (as herein recognized) may be subspecifically related, since he was unable to allocate certain specimens from the Highlands and Brevard areas to either *clemsonae* or *metcalfi*. The distributions also suggest such an arrangement. However, it is probably desirable not to add a new combination of names to the literature until after a study of fresh material has definitely demonstrated morphological intergradation between the two forms.

At the present time, then, it appears best to consider the forms of the Metcalfi Group as allopatric species which replace each other in different mountain ranges. However, it does not seem unlikely that, among the future series of contributions to the knowledge of this group, there will be a demonstration of intergradation between *clemsonae* and *metcalfi*, a demonstration of intergradation between *clemsonae* and *shermani*, and an introduction of a new member of the group from the western part of the range.

Plethodon metcalfi Brimley

Plethodon metcalfi is herein not regarded as being as inclusive as the *metcalfi* of Dunn (1926: 148), Bailey (1937: 6), Stejneger and Barbour (1943: 18), and Bishop (1943: 264). These investigators pictured *metcalfi* as a salamander that occupied a large portion of the Southern Blue Ridge Province. It seems more likely to Mr. Netting and myself, however, that *metcalfi*, as just outlined, is composed of at least two forms. In the complex, at present, there may be readily recognized a small

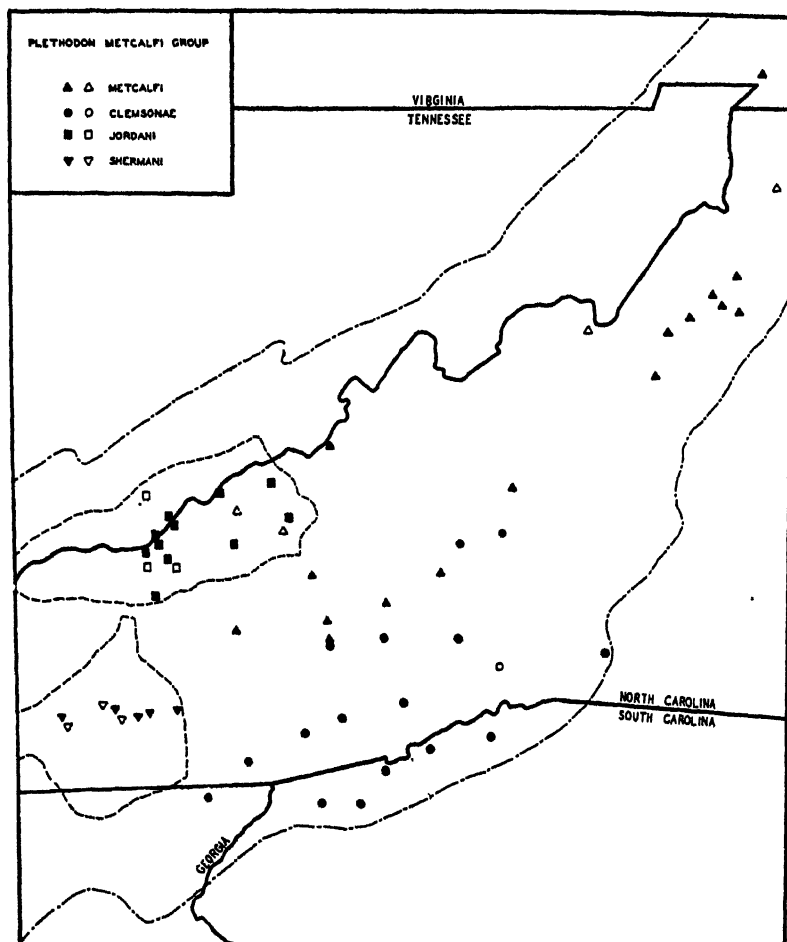


FIGURE 5. Map showing the distributions of the salamanders of the Metcalfe Group of *Plethodon*. Solid symbols represent spot localities based upon museum material; hollow symbols, upon literature reports. The dot-dashed outline is of the Southern Section of the Blue Ridge Province. The area bounded by the broken line along the Tennessee-North Carolina boundary is the Great Smoky Mountains. The broken line surrounding the *shermani* records is discussed in the text.

paler-bellied northern form (for which we propose to restrict the name *metcalfe*) and a large dark-bellied southern form (which will be treated under *clemsonae*).

Whether or not the name *metcalfe* is correctly associated with the small pale-bellied form depends on the identity of the type. It does seem that Brimley's type was an example of the light-bellied form for, in his original description (1912: 138), he wrote, "Bluish plumbeous

above, pale grayish below, the two colors meeting somewhat abruptly on the median line of the sides. Under the lens the pale color of the lower parts . . . From *P. glutinosus* this species is distinguished by . . . the paler underparts." However, the type locality, Sunburst, Haywood County, North Carolina, appears to be in an area where the ranges of the pale-bellied and dark-bellied forms meet; three topotypic specimens in the SCB collection that were collected by Brimley are dark-bellied. Mr. Netting has, as a help toward a clarification of the problem and at least a temporary stabilization of the nomenclature, re-examined the type of *metcalfi* (USNM 5766) and compared it with a typical example (CM 17707a) of the pale-bellied form from Pisgah Mountain, Haywood County, North Carolina. In a letter, under the date of April 14, 1943, he has written in part:

"I believe that the name *metcalfi* should be retained for the light-bellied form. . . . Body slender; size moderate. Head and tail now faded to purplish brown; trunk black, but not the blue black of fresher CM 17707a. Entire lower surface immaculate light brown (this is the brown shade frequently evident in long preserved salamanders and often develops in specimens which had cream-colored venters in life); throat and chest lighter than belly, but uniform in color and without any white spots. Under magnification the venter exhibits hundreds of irregular-sided dark spots, separated by more or less circular whitish areas; the number of spots increases steadily from chin to tail; and the spots are quite similar in form and arrangement to those of CM 17707a, although somewhat fewer in number. Although USNM 5766 now has a browner venter, in general tone, than the Carnegie specimen it exhibits a somewhat sharper line of demarcation between dorsal and ventral coloration on the sides. In my opinion the two specimens are conspecific."

Bailey (1937: 6) has noted two different forms of *metcalfi* (*sensu lato*) and has endeavored to correlate the size and color variation of these with altitude. He wrote, "At lower altitudes the species attains a much greater size. Density of pigmentation is directly correlated with size; at higher altitudes *metcalfi* is both paler and smaller." We have found that there is an almost complete geographical separation of *metcalfi* (*sensu stricto*) from the dark-bellied form (FIGURE 5); it would seem that the assignment of these differences to altitudinal variation is an over-simplification of the situation. That the factors involved are more complex than altitude alone is suggested by the following series which reverse the trend mentioned by Bailey. There is a series (CM 17790) of 25 large specimens of the dark-bellied form which

was collected on Rabun Bald Mountain, near the parking lot, in Rabun County, Georgia, at an altitude of 4000 feet. Another series (CM 17812) of 47 dark-bellied individuals was collected in a nearby ravine at 3500 feet. In contrast is the series of *metcalfi* (*sensu stricto*) (CM 6314) from Boone Fork on the Yonahlossee Road, Watauga County, North Carolina, at 3500 feet. I think it would be more advisable, until the problem of altitudinal variation is carefully studied, to consider these two forms as ecological equivalents of each other as are *Opheodrys aestivus* and *O. v. vernalis* (*vide* Grobman, 1941a: 13) and also *Plethodon dorsalis* and *P. c. cinereus* in the Smoky Mountain Region.

A specimen (MCZ 7226) identified as *metcalfi* is labeled as having been collected in Knox County, Indiana. This is, obviously, an erroneous locality record for this species.

It is perhaps easiest to describe the range of *metcalfi* by saying that it occupies most of the Southern Blue Ridge Province east and north of the ranges of *clemsonae*, *jordani*, and *shermani* (FIGURE 5). Fennemman (1938: 175) is inclined to regard the mountain ranges in the northwestern portion of this province, including the Great Smoky, Chilhowee, Stone, Bald, Holston, and Iron Mountains, as belonging to the Unaka group. The moderately defined ridge along the southeastern and eastern border of the province is the Blue Ridge (*sensu stricto*). Following this terminology, the range of *metcalfi* might be described as follows: altitudes between 3000 and 5800 feet on ridges of the Unakas northeast of the Great Smokies, on ridges of the Blue Ridge (*sensu stricto*) of northern North Carolina and southern Virginia, and on the northwestward-south-eastward running divides connecting the Unakas with the southeastern Blue Ridge (*sensu stricto*). King (1939: 553) mentions two localities for *metcalfi* in the Great Smokies (indicated by hollow triangles on the map) and suggests that migration into the area has been along the divides just mentioned. This form has not been found west of Sylva, Jackson County, North Carolina (UMMZ 81028), but Netting (in a letter of February 13, 1943) suggests that *metcalfi* may range westward through the corridor between the ranges of *shermani* and *jordani* into the Unaka Mountains proper. The thesis herein adopted follows Bailey's suggestion (1937: 8) that *metcalfi* does not cross the Little Tennessee River.

King (1939: 553) has taken *metcalfi* and *jordani* within a mile of each other on the same ridge and is well-convinced of their specific distinctness.

The relationship of this form with *shermani* and *clemsonae* is treated under those forms.

Plethodon clemsonae Brimley

Plethodon clemsonae includes the dark-bellied populations of the Metcalfi Group (other than *shermani* and *jordani*) that occur in the Southern Blue Ridge Mountains south of the range of *metcalfi*. This arrangement allocates to *clemsonae* certain material formerly placed in *metcalfi* and topotypic *clemsonae*.

It is quite likely that *clemsonae*, as herein recognized, is polytypic. Netting writes (in a letter of February 13, 1943) that specimens from the higher altitudes of the Blue Ridge, e.g., Caesar's Head, South Carolina, have black bellies, as compared with the brownish bellies of lowland *clemsonae*. The type of *clemsonae*, which has recently been examined by Netting, is one of the lowland examples (*ca.* 1000 feet) and has a partially brownish, rather than a black, venter. There are lowland *clemsonae* from the valleys and northern slopes of the Southern Blue Ridge and they are thus geographically intermediate between light-bellied *metcalfi* to the northeast and black-bellied high altitude *clemsonae* to the southwest. Various interpretations are possible. It may be that the brown-bellied forms are intergrades between *metcalfi* and high altitude *clemsonae*. If so, three possible nomenclatorial arrangements could be envisaged: (1) The definition of *metcalfi* could be extended to include the intergrades; *clemsonae* would become a synonym of *metcalfi*; and the high altitude black-bellied form would be without a name. (2) The definition of the high altitude black-bellied form could be made to include the brown-bellied intergrades (*vide* Dunn, 1937: 4); thus, there would be two forms, *metcalfi* and *clemsonae*. Or (3) Three forms could be recognized and a new name proposed for the black-bellied populations, restricting *clemsonae* to the brown-bellied intergrades. Of the three alternatives, perhaps the second is to be preferred, since it does not involve the introduction of an additional name into the literature, since bringing together the black-bellied and brown-bellied forms is probably more desirable than combining the brown-bellied and light-bellied populations, and since a nomenclature would not be established before the completion of the ecological study indicated immediately below.

It is also conceivable that the variation in pigmentation of the venter in *clemsonae* (*sensu lato*) is an ecological expression and that high altitude *clemsonae* are black-bellied and that low altitude *clemsonae* are brown-bellied. If this is so, it contradicts the direction of the ecological correlation with density of pigmentation suggested for members of the group by Bailey (1937: 6).

The best procedure indicated for the present, then, is to use the name

clemsonae for the dark-bellied populations of the Southern Blue Ridge. Erroneous records from west of the range are discussed by Bailey (1937: 8).

Three of the four members of the type series were described by Brimley (1927: 74) as having white spots on the back, and at least two other topotypes are recorded (Bishop, 1941b: 20) as possessing dorsal whitish markings. Whether this condition is to be found only in specimens from the vicinity of Jocassee, South Carolina, or in material from more distant areas as well, will be known only after series of fresh material are studied. The freshness of the material may be an unusually important factor in this study. The type specimen, collected for Brimley on April 8, 1927, was described by him in the same year with the words, "... a double row of faint roundish, rather sharply defined grayish spots on the back." On April 10, 1943, Netting, examining the same specimen, wrote, "Jet black above with absolutely no light spots visible." Under certain conditions of preservation (i.e., fixing in 5 per cent formalin and storing in 60 per cent alcohol), this loss of pattern is not immediate. A topotype in the SCB collection, preserved shortly after it was collected on April 8, 1941, still showed quite clearly the light-grayish markings on the back and sides on September 24, 1943.

Plethodon jordani Blatchley

Plethodon jordani is apparently restricted to the Great Smoky Mountains and should be looked for in the western Smoky Mountains (FIGURE 5).

The relationship of this form with *metcalfei* is discussed under that species.

A specimen of *jordani* (AMNH 45298) bears the locality data, Franklin, Macon County, North Carolina. This locality is outside the range of *jordani* and well within the range of *shermani*. Dr. James A. Oliver has recently (April 20, 1943) written to me concerning this specimen as follows: "However, you are probably right in assuming the locality to be in error. The particular specimen sat around the Museum for at least seven years before it was catalogued, also there is no collectors name for this particular entry."

Plethodon shermani Stejneger

Plethodon shermani seems to be a salamander of the Nantahalas. Its range has recently been defined by Bailey (1937: 5) and the broken line on the map (FIGURE 5) represents the river boundaries he has recognized.

Bailey (*ibid.*) has mentioned occasional individuals that possessed lateral white pigment flecks. He also described specimens (both with and without white pigment) in which the red on the legs was reduced in extent. In 1928, Dr. Bishop and his companions collected ten salamanders that were more or less variable with respect to the white pigment and the red limb markings (Bishop, 1941b: 18). These specimens were described and, as regards particular characters, were designated as either closer to *shermani* or closer to *glutinosus*. On the basis of several characteristics mentioned, the following scheme could have been erected for these ten specimens:

Characters in which they were:

1. Close to *glutinosus*: none
2. Intermediate but closer to *glutinosus*: ground color of dorsal surface and head shape
3. Intermediate: ventral coloration
4. Intermediate but closer to *shermani*: none
5. Close to *shermani*: number of vomerine teeth.

On the basis of the information contained in this paragraph, Bishop considered *shermani* a subspecies of *Plethodon glutinosus*.

The relationship may be further considered under three headings, i.e., genetic, morphological, and geographical.

Genetic.—In the case of two plethodontid salamanders of different races of the same species (*Pseudotriton ruber nitidus* and *P. r. schenckii*) that possessed similar but abnormal patterns, it has been suggested (Grobman, 1941b: 179) that mutation of corresponding genes (or some other equal chromosomal aberration) might have been involved in the production of the observed patterns. Moore (1943: 3) has shown that the spotted dorsal patterns of the frogs, *Rana pipiens*, *R. sphenoccephala*, and *R. areolata* are probably controlled by corresponding genes. It may well be that the presence or absence of white pigment in *Plethodon* is controlled by a pair of alleles. In this case, the universal presence of the pattern in *glutinosus* from this area (but *vide* under *glutinosus*) might reflect a very high frequency of one of the alleles; in *shermani*, the occasional occurrence of the pattern may be a result of a lower frequency of this allele. "The same mutant may be a rare aberration in one species, a common phase in another, a species character in a third, and even a generic character elsewhere." (Dobzhansky, 1941: 63.)

Morphological.—There are two types of morphological characters listed by Bishop in describing the ten specimens in question: one is meristic in nature, the other concerns color and shape. Of the latter characters, Bishop has considered his salamanders to be more or less intermediate between *glutinosus* and *shermani* with regard to the density of pigmentation of the venter and of the ground color of the dorsal surface. The individual variation of such characters is often quite extensive. The density of coloration may be affected by the length of time in the preservative; by the preservative itself; may be correlated with ecological conditions; and may change ontogenetically. Many of these same qualifications may apply to characters dealing with head shape.

The reduction of the amount of red pigment on the limbs in those individuals with white markings is not necessarily significant, for Bailey (1937: 5) records reduced red markings for salamanders without white pigment.

The number of vomerine teeth, it would seem, is the most reliable character of those mentioned by Bishop. He found, in material from the area in question, that the average number of vomerine teeth on both sides was 11.6 for 9 *glutinosus*, 8.1 for 9 *shermani*, and 8.4 for the 10 "intergrades." The latter, if these counts are at all representative, are indistinguishable from *shermani* on the basis of the number of vomerine teeth.

Finally, *shermani* is definitely a member of the Metcalfi Group (*vide* Dunn, 1926: 148; Brimley, 1927: 73; Bailey, 1937: 7) and intergradation involving it would most likely be with *metcalfi* or *clemsonae*.

Geographical.—Both *shermani* and *glutinosus* occupy the area in which *shermani* occurs. No evidence regarding ecological segregation is available. This distributional pattern is usually associated with distinct species. Forms which have not yet developed biological reproductive isolation must be geographically or ecologically segregated in order to maintain their separate identities. It remains to be determined whether or not *shermani* is subspecifically related to either *metcalfi* or *clemsonae*.

Perhaps no single item from the above discussion would be of great enough significance to warrant the rejection of the concept of a subspecific relationship between *shermani* and *glutinosus*. It does seem, though, that the cumulative effect of the several bits of evidence and suggestions would be sufficient to sanction the present recognition of *shermani* and *glutinosus* as distinct species.

The Cinereus Group

The Cinereus Group of *Plethodon* comprises the following four forms:

Plethodon cinereus cinereus (Green)

Plethodon cinereus angusticlavius n. subsp.

Plethodon cinereus serratus n. subsp.

Plethodon dorsalis Baird

The group consists of small salamanders with 16 to 19 costal grooves, a yellowish to reddish dorsal band (not apparent in melanistic individuals), and usually without flecks in the pattern. This combination of characters will distinguish the Cinereus Group from the other groups of *Plethodon*. The four forms can be readily identified on the basis of the dorsal pattern when it is present. In addition, *dorsalis* can be distinguished from the *cinereus* races on the basis of several other morphological characters involving the number of costal grooves, the proportionate length of the tail, and the degree of ventral pigmentation. The major apparent differences among the *cinereus* races are those of the shape of the dorsal band. The accompanying figure represents, diagrammatically, the typical pattern of each form (FIGURE 6).

The group is widespread over the eastern United States (FIGURES 7 and 10) and may be described as consisting of two partially allopatric species, one of which, *cinereus*, is polytypic. The races of *cinereus* replace each other geographically and are apparently reproductively isolated from each other by physical barriers. The gap between *serratus* and *angusticlavius* seems to be the broad plain of the Arkansas River Valley. The factors responsible for the isolation of *angusticlavius* from *cinereus* may be the low relief and paucity of hardwoods (where trees are present the association is an oak-pine) of the Salem Plateau in southeastern Missouri. Although over half of the circumference of the *dorsalis* range includes portions of the range of *cinereus*, the former occupies an area that is physiographically different from the greater part of the range of the latter. Intergrades between the two species are not known from any area including that in which the spacial relationship between the two is sympatrical.

The evidence culled from literature accounts and from limited personal field experience indicates that the members of this group are to be found in wooded areas where the bark and fallen logs afford ample cover. Further, the drier Midwestern areas must have relief enough, at least, to afford available retreats in which the moisture conditions remain above the threshold requirements of the salamanders. If the two factors, to which reference has just been made (relief and wood-

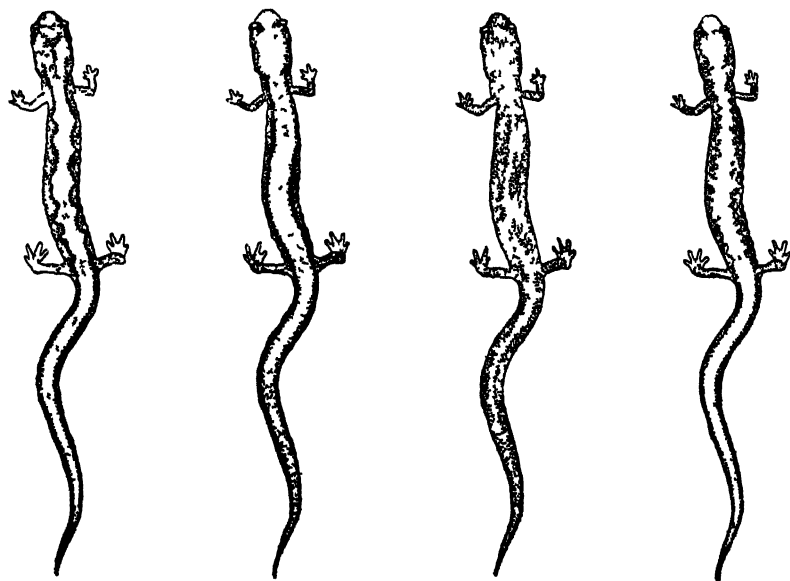
**DORSALIS****CINEREUS****ANGUSTICLAVIUS****SERRATUS**

FIGURE 6. Diagrammatic sketches illustrating the typical patterns of the members of the Cinereus Group of *Plethodon*. The drawings of *angusticlavius* and *serratus* were made from the holotypes. Hugh P. Christ, delmeistor.

lands), are of importance in controlling the distribution of the salamanders of the Cinereus Group, then a high correlation should exist between the areas so characterized and the ranges of the salamanders. That such a correlation is possible is demonstrated by the accompanying map (FIGURE 7), deviations from the required pattern are dealt with under the separate species accounts.

Whether the pattern of *cinerens* was derived from that of *dorsalis*, as suggested by Dunn (1926: 24), will not be considered here, but I am in accord with his statement from the same page: "The *cinerens* group has no obvious center, being perhaps, the local differentiations

FIGURE 7. Map showing the distributions of the race of *Plethodon cinereus*. Solid symbols represent county localities from which museum material has been available; hollow symbols, additional literature records. The eastern, southern, and western (except that section which includes the *angusticlavius* range) borders of the eastern forest area are approximated by the dot-dashed line. This represents the actual border of the area of forests (including the northern coniferous, north-eastern hardwood, northwestern pine, and southern hardwood forests), but excluding the river bottom, southeastern pine, and subtropical forests, and was adapted from the Map of Natural Vegetation of the United States (Shantz and Zon, 1924: 5). The dotted line delimits the relatively heavily dissected hardwood forested southern section of the Ozark Plateaus. The broken line bounds the Ouachita Mountains and the higher parts of the adjacent Arkansas Valley, south of the Arkansas River.

of a single original form, as they [*cinereus* and *dorsalis*] have different ranges" It does not seem improbable that the differentiation between *cinereus* and *dorsalis* may have been favored by an isolation arising from an ecological segregation in the mountains (probably in the Southern Blue Ridge, whence, Dunn believes, the entire family was dispersed) If the isolation were complete, the degree of differentiation

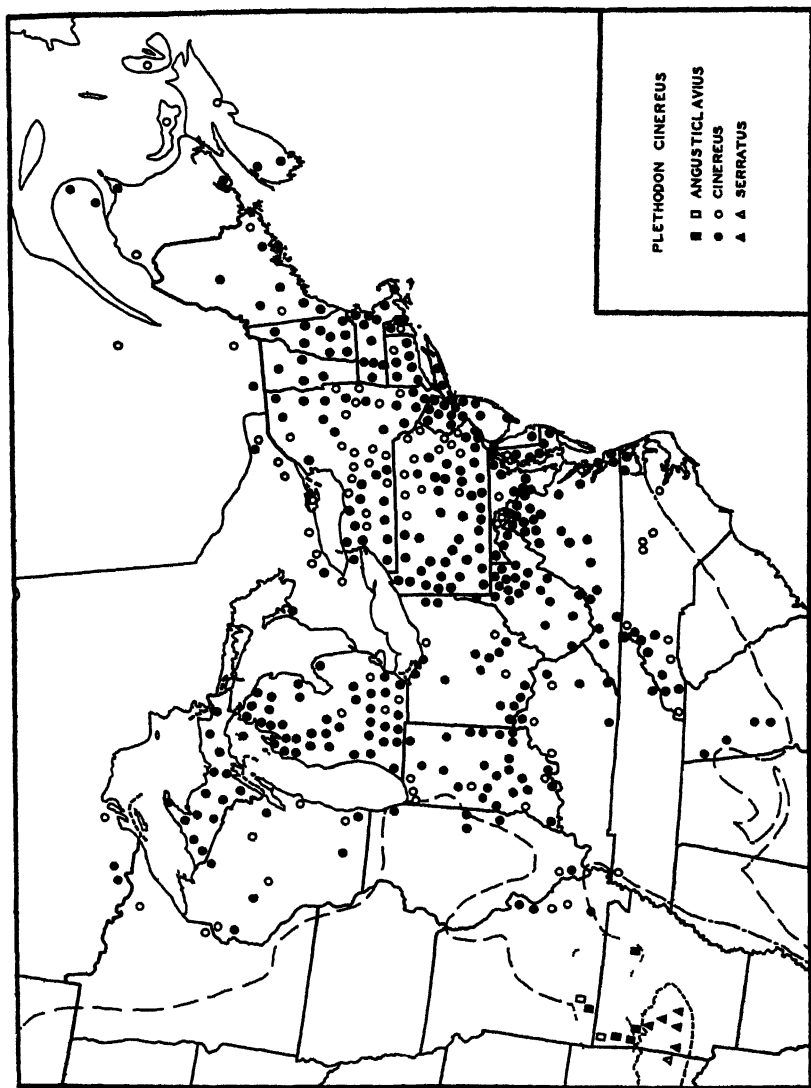


FIGURE 7

would then depend upon the composition of the breeding populations, random fixation of genetic changes, mutation rates, selection, and the time involved. These processes presumably could produce differences of various sorts, including others than the observed morphological ones. Should any of these differences, or combinations of them, prove sufficient to produce reproductive isolation, the forms could then meet again and maintain the characteristics that had been evolved. The habitats of low altitude *dorsalis* (as it occurs in the Blue Ridge) and those of high altitude *cinereus* (as it is found in the Blue Ridge) apparently merge in southern Indiana, among other localities. Here, both forms are found in the same ravines with no intermediacy of characters. There is no conflict with the evidence if one considers these forms as allopatric species with spreading ranges that are merging. Such a condition, in the absence of strong inter-species competition, might result in the formation of a sympatrical species relationship between *dorsalis* and *cinereus*.

Of the two, *cinereus* is the more widely distributed, and it (or its prototype) was able to spread southwestward into the areas now occupied by *serratus* and *angusticlavius*. The isolation of the populations in these areas from the main body of the *cinereus* (or proto-*cinereus*) populations may be accounted for in at least three ways: (1) the efficiency of the natural barriers as effective isolating agents developed subsequent to the arrival of the *cinereus*-stock into the area; (2) the effectiveness of the barriers was relatively constant and these barriers were such as to permit the passage from one area to another of relatively small numbers of individuals which were insufficient to swamp the differentiation that was occurring; and (3) a combination of 1 and 2. As already suggested, the isolation promoted differentiation. It seems more likely that this has been the general manner of the evolution of *serratus* and *angusticlavius* rather than the chain method (i.e., *cinereus* → *angusticlavius* → *serratus*), since the patterns of both *serratus* and *angusticlavius* appear to have been directly derived from *cinereus* rather than in the manner indicated by the chain method.

Plethodon cinereus cinereus (Green)

The range of this salamander is the most extensive of any member of the *Cinereus* Group (FIGURE 7). Its relationships with *P. c. angusticlavius*, *P. c. serratus*, and *P. dorsalis* are discussed under those forms. Since several localities, represented either by museum specimens or literature reports, are thought to be associated erroneously with *cinereus*, they are discussed below.

One salamander (ANSP 4346) from Bellevue, Davidson County, Tennessee, is unrecognizable, being shrunken and faded. It may be *dorsalis*.

Several specimens (AMNH 35517-9) are labeled, Lakeland, Georgia. If this is the Lakeland of Lanier County, it is quite far removed from the nearest other record and should be verified before being accepted.

A Hurter specimen (USNM 57106) is listed from Franklin County, Tennessee. Judging from other available information, however, it would seem that this region is one inhabited by *dorsalis* and not by *cinereus*. But *cinereus* probably does occur in Franklin County, Missouri, which is adjacent to St. Louis County, where Hurter lived and had himself collected *cinereus*. I have made this same suggestion (Grobman, 1941a: 15) previously, with regard to specimens of *Opheodrys vernalis blanchardi* which, allegedly, were collected by Hurter in Franklin County, Tennessee, but which, if actually collected in a Franklin County, were probably from the Missouri one. This record is therefore not mapped for *cinereus* in Tennessee and, pending more definite information, is not included in Missouri, either.

Pickens (1927: 107) included this form as occurring at Camden, in his list of the amphibians of upper South Carolina, on the basis of the statements by DeKay (1842: 75) and Holbrook (1842: 44) to the effect that Dr. Blanding had informed these authors that he had seen this form at Camden. For over 100 years this sight record has, apparently, not been verified.

Cope and Packard (1881: 878) record *cinereus* from Nickajack Cave, Marion County, Tennessee. This was previous to Cope's (1889: 138) publication of a description of *dorsalis*, although he had used the name *dorsalis* prior to 1881 (Cope, 1869: 100). By a comparison of the available distributional data it would seem that Cope and Packard had a specimen of *dorsalis* rather than *cinereus*. This locality is not included on either the *cinereus* or *dorsalis* map.

On the other hand, one record for *cinereus* is included for Obion County, Tennessee (represented by a hollow circle on the map), on the strength of a report by Parker (1939: 75) about *Plethodon dorsalis*. Under the latter heading, he wrote, "The coloration characteristically includes a light-colored zigzag or straight dorsal stripe on body and tail." It would seem likely, then, that he had obtained both *cinereus* and *dorsalis* in the area.

Typical *cinereus* and *dorsalis* co-inhabit the eastern states that originally had a forest cover. The number of instances of the spilling out from this area by *cinereus* is not large. It remains to be determined

whether the southern portion of the bounded area is inhabited by *cinereus* (Piedmont of South Carolina and adjacent Georgia and adjacent North Carolina) and *dorsalis* (northern Mississippi and southwestern Tennessee). It may be found, in the case of the latter, that *dorsalis* does not have an extensive range in the coastal plain, material from this area being known only from Reelfoot Lake, Obion County, Tennessee (UMMZ 84364).

The areas of Kentucky and Tennessee within the boundary and devoid of *cinereus* records are, for the most part, populated by *dorsalis* (FIGURE 10).

Plethodon cinereus occurs farther north than the map suggests. An indication of this is a specimen (USNM 5963) bearing the locality data, Hudson Bay Territory.

Several series from Indiana (e.g., UMMZ 72387) contain a high proportion of melanistic and large individuals. The coastal groove count is that of *cinereus* (these specimens are not *richmondi*) and, superficially at least, there are no striking morphological differences between these populations and those from other parts of the *cinereus* range.

Plethodon cinereus angusticlavius NEW SUBSPECIES

Holotype.—AMNH 40366, adult male, collected at Mud Cave, near Fairy Cave, Stone County, Missouri, on October 1, 1927, by B. C. Marshall.

Paratypes.—AMNH 40367, UR 7122, 7124–6, topotypes; UR 7121, 7132–5, Locust Grove, Independence County; UR 7120, 7127, Batesville, Independence County; and CM 21960, UR 7128–9, 3 miles south of Locke, Crawford County; all in Arkansas; and USNM 57089, Stone County, Missouri.

Additional Material.—FMNH 39593, Franklin County, Arkansas; FMNH 26402–5, 6 miles east of Springdale, Washington County, Arkansas; USNM 49959, 57088–9, Stone County, Missouri; UAM, 3 miles southwest of Locke, Crawford County, Arkansas; and UAM, Winslow, Washington County, Arkansas.

Diagnosis.—A *Plethodon* closely related to *P. c. cinereus* and differing from it most conspicuously in the possession of a narrower dorsal band with indistinct edges.

Description of the Type Series.—Of the seventeen specimens in this series, two have not had their measurements included, because their dorsal bands are somewhat obscured, and two others are sub-adult individuals which have been excluded, since the extent of ontogenetic

variation in the character under consideration has not been determined (*vide* Grobman, 1943: 11). These four paratypes can, however, be readily distinguished from individuals of *c. cinereus* and *c. serratus* by the narrowness of their dorsal stripes.

With the aid of an ocular micrometer the following measurements were recorded from the remaining thirteen specimens: the width of the body, midway between the fore and hind limbs, and, at the same region, the greatest width of the dorsal band. These measurements were also made on three non-paratypical *angusticlavius* and on several series of *cinereus*: UR 1193 (20), Rochester, New York; UMMZ 72387 (5), Cass County, Indiana; UMMZ 88547 (8), Mt. Holly, New Jersey; SCB (2), USNM 57093 (1), 93144 (1), Jefferson County, Missouri; UMMZ 77802 (8), 77803 (1), SCB (1), USNM 24475 (1), 57090-1 (2), 35728 (1), St. Louis County, Missouri; USNM 57092, San Francisco County, Missouri; and UMMZ 77380 (1), 77378 (10), Keeners, Butler County, Missouri (FIGURE 8).

These measurements may be expressed in terms of the percentage of

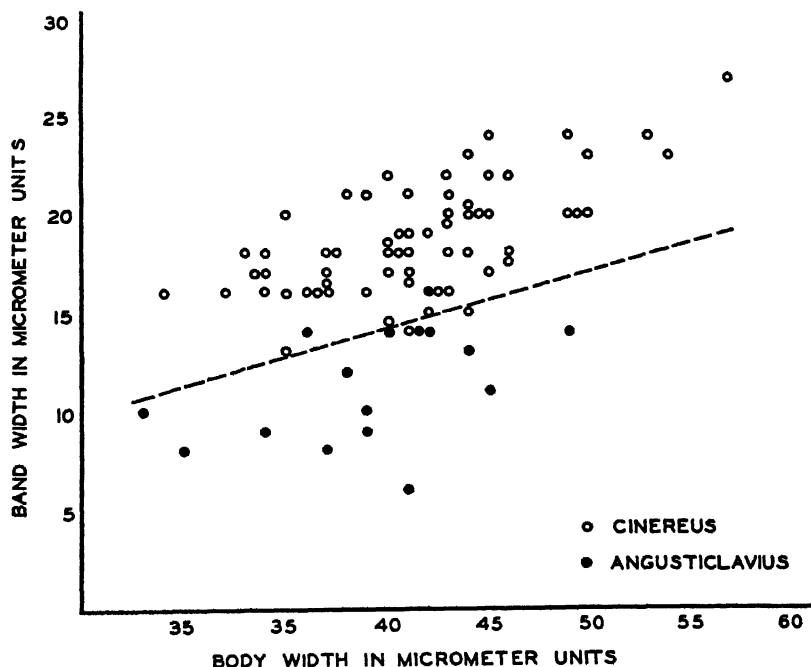


FIGURE 8 Graph showing the relationship between body width and band width in *P. c. cinereus* and *P. c. angusticlavius*. The broken line suggests a division that will correctly separate 95 per cent of the material examined.

the body width that is occupied by the dorsal band. This figure is 28 per cent for 16 *angusticlavius* as compared with 45 per cent for the 63 *cinereus* specimens studied (TABLE 2).

TABLE 2
FREQUENCY DISTRIBUTION OF THE WIDTH OF THE DORSAL BAND MEASURED AS A PERCENTAGE OF BODY WIDTH, MIDWAY BETWEEN THE FORE AND HIND LIMBS, OF *PLETHODON C. CINEREUS* AND *PLETHODON CINEREUS ANGUSTICLAVIUS*

Percentage	<i>cinereus</i>	<i>angusticlavius</i>
13-15	0	1
16-18	0	0
19-21	0	0
22-24	0	4
25-27	0	2
28-30	0	3
31-33	0	3
34-36	4	1
37-39	6	2
40-42	8	0
43-45	16	0
46-48	11	0
49-51	10	0
52-54	4	0
55-57	4	0

Not only is the band narrower in *angusticlavius* but its borders are not as sharply defined as they are in *cinereus*. This occasionally results in a varying width of the band in a few specimens of *angusticlavius*.

No other differences have been noticed between this form and *cinereus*. The costal groove count and the disposition of the teeth seem to be similar to that of *cinereus*. *P. c. angusticlavius* appears to be slightly smaller than *cinereus*.

Remarks.—A series of 12 Arkansas specimens from Washington County (FMNH 26402-5 and UAM (7)) and Franklin County (FMNH 39593) have been tentatively identified as *angusticlavius*. Two of the 12 are melanistic, seven exhibit a good *angusticlavius* pattern, and three have a pseudo-*dorsalis* pattern. The last-mentioned three individuals (FMNH 26404, 39593, and UAM) are small specimens having comparatively narrow bands with irregular serrations of the borders anteriorly. These serrations are not bilaterally symmetrical and do not coincide with the costal grooves. These specimens may be indicators of intergradation between *serratus* and *angusticlavius* in this area. One of these specimens (FMNH 26404) is doubtless

the individual upon which was based the Arkansas record for *P. dorsalis* (Black and Dellinger, 1938: 7).

Whether the relationship between this form and *cinereus* is that between allopatric species or between subspecies can, at present, be decided only by inference. Since it cannot now be determined whether or not *angusticlavius* and *cinereus* are reproductively isolated (other than geographically), the question to be answered is, probably, whether *angusticlavius* is more or less divergent from *cinereus* than are well-established species and subspecies in the *Cinereus* Group. Admittedly, this method of determining systematic rank can lead only to a tentative approximation. Since no race is now recognized that has a range contiguous with *cinereus* and intergrades with it across the common boundary, it cannot be decided whether *angusticlavius* differs more or less from *cinereus* than would an unquestionable race. It does seem quite clear, however, that *dorsalis* and *cinereus*, although closely related, are reproductively isolated full species. (For a discussion of the relationship between these two species see under *dorsalis*.) It is also quite apparent that *angusticlavius* is much more closely related to *cinereus* than is *dorsalis*, inasmuch as it appears to differ from *cinereus* only in one character, whereas the differences between *dorsalis* and *cinereus* are more numerous and, probably, of greater biological magnitude. Since *cinereus* and *dorsalis* are closely related species, it seems that the best estimate of the relationship between *cinereus* and *angusticlavius* is to consider the latter a subspecies of the former.

The name, *angusticlavius*, is from the Latin, meaning "wearing a narrow stripe."

Range.—Besides the material examined (Crawford, Franklin, Independence, and Washington Counties, Arkansas, and Stone County, Missouri), there are literature records of *Plethodon cinereus*, which probably refer to this form, from Benton County, Arkansas (*ibid.*), and Christian County, Missouri (Stone, 1903: 538). These records are indicated by hollow squares on the map.

This form is apparently confined to the relatively heavily dissected, hardwood forested, southern section of the Ozark Plateaus (FIGURE 7). The range of *angusticlavius*, therefore, separates those of *cinereus* to the northeast and *serratus* to the south. Intergradation between *angusticlavius* and *cinereus* is not known, although it should be pointed out that no material has been available from the region around the supposed barrier. Individuals from the easternmost part of the *angusticlavius* range (Independence County, Arkansas) have a stripe that is just as narrow as that of the remaining *angusticlavius* specimens

available, and the dorsal band of most nearly adjacent *cinereus* (Butler, Jefferson, and St. Louis Counties, Missouri) is scarcely different in width from that of typical eastern *cinereus*.

Plethodon cinereus serratus NEW SUBSPECIES

Holotype.—FMNH 39464, female, collected on Rich Mountain, Polk County, Arkansas, at an altitude of 2500 feet, on March 23, 1938, by Karl P. Schmidt and C. M. Barber.

Paratypes.—FMNH 28431, topotypes; FMNH 28391–2, north side of Mt. Magazine, at 2400 feet, Logan County; FMNH 28430, base of Rich Mountain, at 1800 feet, Polk County; CM 21961, UR 7123, 7130–1, Mt. Nebo, six miles west of Dardanelle, Yell County; UMMZ 77806, 77379, Rich Mountain, Polk County; UMMZ 77805, 77808, near Acorn, Polk County; UMMZ 77804, Garland County; UMMZ 83298, Caddo Gap, Montgomery County; and SCB, halfway up north side of Rich Mountain, Polk County; all in Arkansas.

Diagnosis.—A *Plethodon* closely related to *P. c. cinereus* from which it differs most conspicuously by its possession of a narrow dorsal band with saw-toothed edges in which the serrations coincide with the costal grooves.

Description of the Type Series.—Of the 61 specimens of *serratus* available for study, eight are melanistic. Of the remaining 53, 45 (or 85 per cent) can be readily identified by means of the dorsal pattern. In these, the lateral margins of the dorsal stripe are not straight, as in *cinereus*, but serrated. The serrations coincide with the costal grooves and, in meeting their dorsal ends, form a pattern in which the dorsal band is alternately wide and narrow. The band is widest at the grooves and narrowest at the middle of the folds. This pattern can be more readily derived from a *cinereus* pattern than from a *dorsalis* pattern.

The venter of *serratus*, especially in the gular region, is somewhat more pigmented than that of *cinereus* from localities in New York and Michigan, but not greatly different, if at all, from *cinereus* collected in New Jersey, North Carolina, and Indiana.

The number of costal grooves seems to be the same as that of *cinereus*.

The head is relatively narrower (FIGURE 9) in 60 *serratus* than in 211 *cinereus* (29 from Linville, North Carolina; 38 from Cass County, Indiana; 96 from Rochester, New York; 22 from Mt. Holly, New Jersey; and 26 from Allegan County, Michigan). In the 41–45 mm. head-trunk length class, for example, the head width of *serratus* is 43.0 mi-

chrometer units, as compared with 47.4 in the *cinereus* sample. This difference is statistically significant, inasmuch as it contains its standard error well over nine times. However, there is considerable variation among the several *cinereus* populations; the sample from Indiana, for example, has a head-width growth curve almost identical with that of *serratus*, both of these differing widely from the curves of the New York and North Carolina samples.

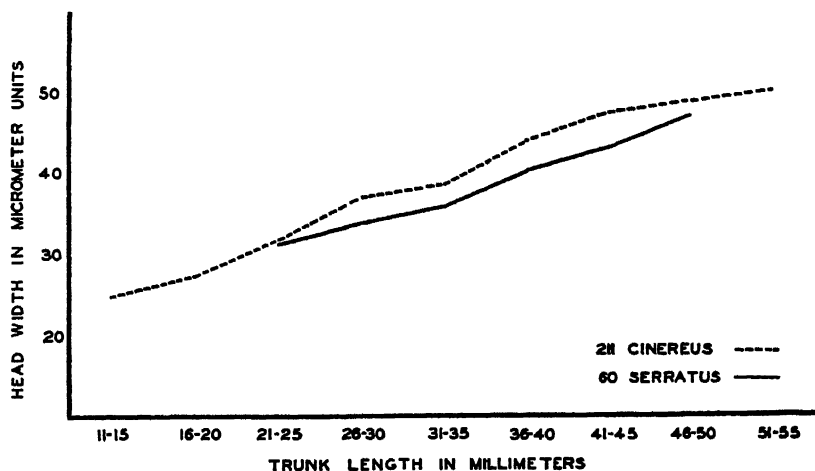


FIGURE 9 Graph illustrating the relationship between head-trunk length and head width in samples of *P. c. cinereus* and *P. c. serratus*.

In small samples that were considered representative, the number of vomerine teeth per side was found to be 5.1 in *serratus*, 5.3 in *dorsalis*, and 5.5 in *cinereus*. The differences between these means are probably largely reflections of random sampling errors. Nor does the disposition of these, or the parasphenoid teeth, in *serratus* seem to be different from that found in *dorsalis* or *cinereus*.

P. c. serratus appears to be somewhat smaller than *cinereus*.

Remarks.—This form is considered a subspecies of *cinereus*, because the problem involved is essentially similar to that with which the assignment of systematic rank was concerned in *angusticlavius*. The relationship between *serratus* and *angusticlavius* is discussed under the latter form.

The name, *serratus*, refers to the condition of the diagnostic character: the saw-toothed edge of the dorsal band.

Range.—The material upon which the description is based is from the following Arkansas counties: Garland, Logan, Montgomery, Polk,

and Yell. Dunn and Heinze (1933: 121) reported *cinereus* from the Oklahoma side of Rich Mountain, so it seems that *serratus* can also be recorded from Le Flore County, Oklahoma (FIGURE 7). All the localities for *serratus* are from within the Ouachita Province and it seems likely that this race is confined to the Ouachita Mountains and the higher parts of the adjacent Arkansas Valley south of the Arkansas River. This is a region originally supporting a rather heavy growth of conifers and hardwoods.

Plethodon dorsalis Baird

Besides the records from museum material and the literature that have been plotted (FIGURE 10), *Plethodon dorsalis* has been reported from Franklin, Venango County, Pennsylvania (Cope, 1892: 964), and from Salem, Essex County, Massachusetts (Cope, 1869: 100). These records are undoubtedly in error. Black and Dellinger (1938: 7) list under this name a specimen that was collected near Springdale, Washington County, Arkansas. This specimen apparently is an example of *Plethodon cinereus angusticlavius* which is described in this paper.

The greater area of the range of *dorsalis* is within the Interior Low Plateaus Physiographic Province. *P. dorsalis* occurs farther to the north of this area in Indiana, south of the plateaus in Alabama, and east and west of them in Tennessee.

Cope (1869: 100) listed this form as a variety "dorsalis, Baird, M. S. 3776" of *cinereus*. Stejneger and Barbour (1917: 15) pointed out that therein *dorsalis* was a *nomen nudum*, since a description did not accompany this first printed appearance of the name. These authors then recognized the account in the "Batrachia" (Cope, 1889: 138) as the original description of the form and accredited the name to Cope. Cope, however, had headed this description as follows: "*Plethodon cinereus dorsalis* Baird." He then cites his 1869 paper and follows the citation with a parenthetical remark, "name only." It appears reasonably clear to me, therefore, judging from this "original account," that Baird was most likely responsible for the name and description.

The relationship between this form and *cinereus* has been variously treated. Cope (1869: 100, *et seq.*) considered the relationship between the two forms to be subspecific, but Dunn (1926: 162) regarded them as distinct species, since he had seen series of both forms from several localities where they had been found occurring together. Blanchard (1925: 369) considered the relationship to be subspecific, largely on the basis of a series of specimens collected near Nashville, Brown County, Indiana. In this series, there are five specimens

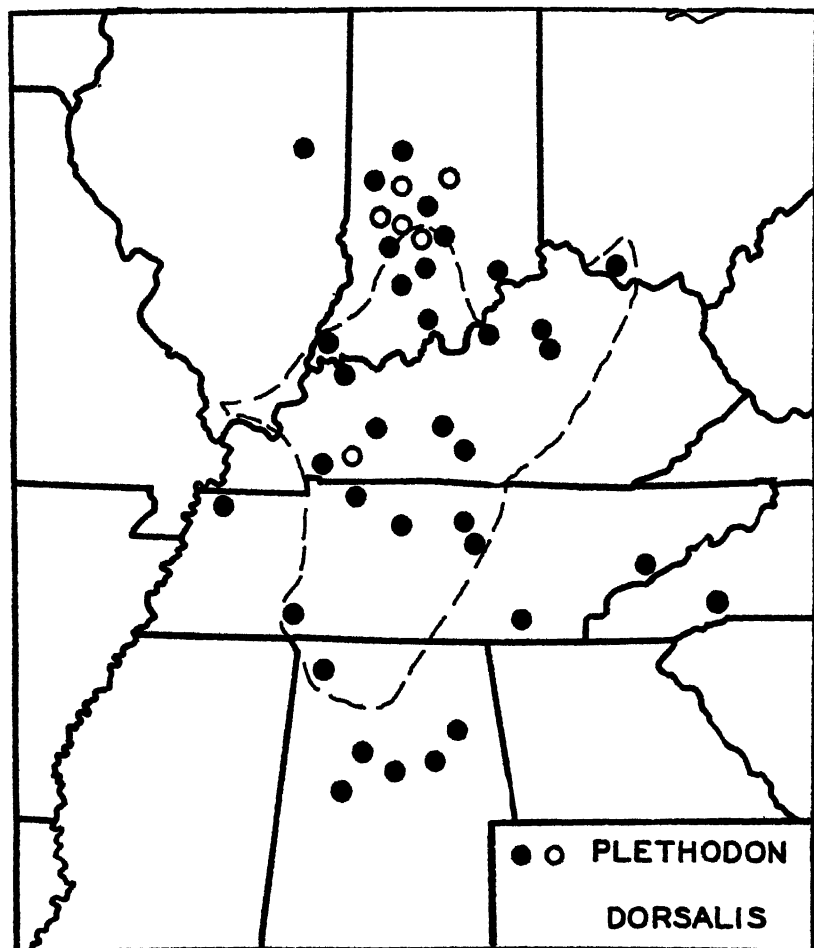


FIGURE 10 Map showing the distribution of *Plethodon dorsalis*. The solid circles represent county localities of available museum specimens and the hollow circles, of additional literature records. The broken line represents the boundary of the Interior Low Plateaus Physiographic Province.

(UMMZ 60992) that he regarded as *cinereus*, one (UMMZ 60990) that he considered *dorsalis*, and one (UMMZ 60991) that he listed as combining the patterns of *cinereus* and *dorsalis*. In this particular individual the anterior part of the dorsal band is wavy, or lobular, as is the condition of the entire band in *dorsalis*. The posterior portion of the band in this individual has straight parallel borders as does the entire band in *cinereus*. This "intergrade" type of pattern, however, is also to be found in one salamander (UMMZ 77375) from near

Clarksville, Montgomery County, Tennessee. This locality is approximately in the middle of the *dorsalis* range and is about 200 miles from the nearest *cinereus* influence. It would seem, then, that this particular type of "intergrade" pattern is merely one of the variant expressions of the *dorsalis* pattern. The two forms must therefore be regarded as distinct species with overlapping ranges pending the demonstration of intergradation.

In 1939, King (p. 551) first reported *Plethodon dorsalis* from the eastern part of Tennessee in the Smoky Mountains National Park. Since some workers have subsequently expressed skepticism concerning these locality data for *dorsalis*, I have re-examined the material used by King. This material is contained in 46 specimens in the collection of the Great Smoky Mountains National Park. These specimens were taken in the vicinity of the Tennessee side of the Park at elevations below 2200 feet.

They more closely resemble a series of representative *dorsalis* from Woodford County, Kentucky (SCB), than a sample of typical *cinereus* from Linville, North Carolina (UR 4276), at least in the lower costal groove count, a lobulated dorsal band (when discernible), and a comparatively light venter. *Plethodon dorsalis* is apparently a more slender salamander than *cinereus*, but these Smoky Mountain individuals are quite stout-bodied. They also differ from typical *dorsalis* in being paler and in having the dorsal pattern much less evident. It is quite conceivable, however, that these three last-mentioned differences between the Smoky Mountain sample and other *dorsalis* are perhaps due to differences in methods of preservation.

I do not see why these locality data cannot be considered valid. Most of eastern Tennessee immediately west of the Blue Ridge Province is *terra incognita*, as far as salamanders are concerned, and I should expect these *dorsalis* valley populations of the Smoky Mountains to be continuous (at least up to very recently, if not in present times) with those of the *dorsalis* populations from the Interior Plateaus.

Another indication that *dorsalis* occurs in the lowlands of this area is the series of two specimens (CM 16977-8), given to Mr. Netting by Dr. Blanchard, collected by Ethel B. Finster, May 9, 1931, 13 miles south of Asheville, North Carolina. These two salamanders have the *dorsalis* pattern anteriorly but a *cinereus* pattern posteriorly; however, as mentioned above, this pattern is believed to be one of the extremes of the *dorsalis* pattern and not one resulting from intergradation. They also have the lower costal groove number of *dorsalis*. The area from which these individuals came is in the French Broad River valley and

much of this territory is at an altitude of less than 2200 feet, which appears to be the upper limit at which King found *dorsalis* in the Smoky Mountains National Park. It does not seem unlikely that *dorsalis* entered this region from the west, along the French Broad River valley; for this valley, from a point about 30 miles south of Asheville, continues, at an altitude less than 2200 feet, northward and westward into the heart of the range of *dorsalis*.

The region under consideration is, then, another area where the species *cinereus* and *dorsalis* coexist; only here it seems that there is a definite ecological segregation, with *cinereus* occupying the higher altitudes and *dorsalis* the lower river-bottom country (King 1939: 550).

The widest area from which both species have been reported centers about southern Indiana. I have seen collections made at one time and at one place that contained both species from the following Indiana localities: Washington Township, Brown County (CM 13578-93); 2 miles west of Nashville, Brown County (UMMZ 60990-2); near Worthington, Green County (CSNH 2265-6); Fort Ritner, Lawrence County (MCZ 7294-5); McCameron Township, Martin County (CM 13358-9); and Mecca, Parke County (MCZ 7292-3). There is some evidence that ecological segregation may also occur here (Dunn, 1926: 160), but, if so, it certainly is not as marked as in the Smoky Mountains region where the topography is more varied.

The Welleri Group

The following three forms are here assigned to the Welleri Group:

Plethodon richmondi Netting and Mittleman

Plethodon nettingi Green

Plethodon welleri Walker

This group is most closely related to the Cinereus Group and this relationship is discussed under the account of that group. The outstanding unifying characteristic of the Welleri Group is the presence of dorsal greenish golden to brassy flecks which usually disappear in the preservative.

Our knowledge of this group is very recent and has accumulated subsequent to the publication of Dunn's monograph on the Plethodontidae. First attention to a member of this group was focused on *welleri* less than fifteen years ago by Walker, original description (1931: 3). Not until 1938, did the descriptions appear of the other two known members of the group, *richmondi* by Netting and Mittleman (1938: 288), and *nettingi* by Green (1938: 295).

The relationship within the group has been discussed by Green (*ibid.*, pp. 298-9) and little can be added here to that discussion. It seems that *nettingi* and *welleri* are localized montane derivatives of a pre-*richmondi* stock (FIGURE 11). Whether the relationship is that among distinct species or among intergrading geographic races can be determined only after a study has been made of the material from the lower slopes of the mountain ranges inhabited by *welleri* and by *nettingi*.

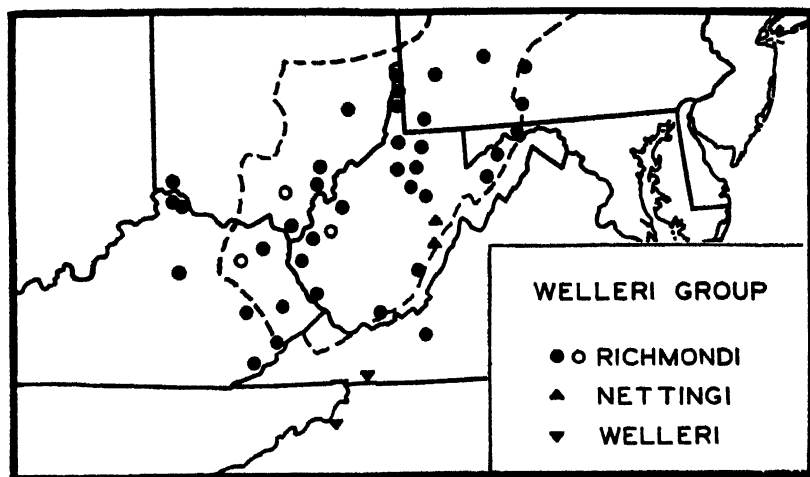


FIGURE 11. Map showing the distribution of the salamanders of the Welleri Group of *Plethodon*. Solid symbols represent county localities based upon museum material; hollow symbols, upon literature records. The broken line bounds the adjacent Allegheny Mountains and Unglaciated Allegheny Plateaus Sections of the Appalachian Plateaus Physiographic Province.

Plethodon richmondi Netting and Mittleman

This is a salamander with its geographic center in the Allegheny Plateaus (FIGURE 11). *Plethodon richmondi* is not confined to this area. It is known from as far west as Independence, Kenton County, Kentucky (BHFM 262; these specimens were listed as *P. cinereus* by Dury and Williams, 1931: 3), and southern specimens from Watauga County, North Carolina (not represented on the accompanying map), are tentatively referred to *richmondi* by Netting and Mittleman (1938: 292).

The range is probably greater than that represented on the map. *P. richmondi* had been known for less than five years and little information concerning its distribution has been published since the original description appeared (Netting and Mittleman, 1938: 287-93). Specimens catalogued as *cinereus* are probably to be found in many of the

major museum collections, and it is not improbable that a study of this material will yield additional distributional information.

Plethodon nettingi Gicen

Plethodon nettingi is apparently restricted in its distribution to the Cheat Mountain Range in Randolph and Pocahontas Counties, West Virginia (FIGURE 11).

Plethodon welleri Walker

This species is known from but two localities: Grandfather Mountain, North Carolina (MCZ 17365-6, USNM 84135, UMMZ 75507, CSNH 776, and UR 4177), and Whitetop Mountain, Virginia (UMMZ 7511 and CSNH 1544). These localities are within the Southern Section of the Blue Ridge Province and are well over 5000 feet in altitude (FIGURE 11).

The Whitetop specimens differ from the topotypic individuals from Grandfather Mountain in a relatively greater spotting of the venter, as has been pointed out by Walker (1934: 190), and in the darker ground color of the venter.

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THE ORGANIZATION OF THE NEW YORK ACADEMY OF SCIENCES

THE ORIGINAL CHARTER

AN ACT TO INCORPORATE THE
LYCEUM OF NATURAL HISTORY IN THE CITY OF NEW YORK

Passed April 20, 1818

WHEREAS, The members of the Lyceum of Natural History have petitioned for an act of incorporation, and the Legislature, impressed with the importance of the study of Natural History, as connected with the wants, the comforts and the happiness of mankind, and conceiving it their duty to encourage all laudable attempts to promote the progress of science in this State—therefore,

1. *Be it enacted by the People of the State of New York represented in Senate and Assembly*, That Samuel L. Mitchell, Casper W. Eddy, Frederick C. Schaeffer, Nathaniel Paulding, William Cooper, Benjamin P. Kissam, John Torrey, William Cumberland, D'Jurco V. Knevels, James Clements and James Pierce, and such other persons as now are, and may from time to time become members, shall be, and hereby are constituted a body corporate and politic, by the name of LYCEUM OF NATURAL HISTORY IN THE CITY OF NEW YORK, and that by that name they shall have perpetual succession, and shall be persons capable of suing and being sued, pleaded and being impleaded, answering and being answered unto, defending and being defended, in all courts and places whatsoever; and may have a common seal, with power to alter the same from time to time; and shall be capable of purchasing, taking, holding, and enjoying to them and their successors, any real estate in fee simple or otherwise, and any goods, chattels, and personal estate, and of selling, leasing, or otherwise disposing of said real or personal estate, or any part thereof, at their will and pleasure: *Provided always*, that the clear annual value or income of such real or personal estate shall not exceed the sum of five thousand dollars: *Provided*, however, that the funds of the said Corporation shall be used and appropriated to the promotion of the objects stated in the preamble to this act, and those only.

2. *And be it further enacted*, That the said Society shall from time to time, forever hereafter, have power to make, constitute, ordain, and establish such by-laws and regulations as they shall judge proper, for the election of their officers; for prescribing their respective functions, and the mode of discharging the same; for the admission of new mem-

bers; for the government of the officers and members thereof; for collecting annual contributions from the members towards the funds thereof; for regulating the times and places of meeting of the said Society; for suspending or expelling such members as shall neglect or refuse to comply with the by-laws or regulations, and for the managing or directing the affairs and concerns of the said Society: *Provided* such by-laws and regulations be not repugnant to the Constitution and laws of this State or of the United States.

3. *And be it further enacted*, That the officers of the said Society shall consist of a President and two Vice-Presidents, a Corresponding Secretary, a Recording Secretary, a Treasurer, and five Curators, and such other officers as the Society may judge necessary; who shall be annually chosen, and who shall continue in office for one year, or until others be elected in their stead; that if the annual election shall not be held at any of the days for that purpose appointed, it shall be lawful to make such election at any other day; and that five members of the said Society, assembling at the place and time designated for that purpose by any by-law or regulation of the Society, shall constitute a legal meeting thereof.

4. *And be it further enacted*, That Samuel L. Mitchill shall be the President; Casper W. Eddy the First Vice-President; Frederick C. Schaeffer the Second Vice-President; Nathaniel Paulding, Corresponding Secretary; William Cooper, Recording Secretary; Benjamin P. Kissam, Treasurer, and John Torrey, William Cumberland, D'Jureo V. Knevels, James Clements, and James Pierce, Curators; severally to be the first officers of the said Corporation, who shall hold their respective offices until the twenty-third day of February next, and until others shall be chosen in their places.

5. *And be it further enacted*, That the present Constitution of the said Association shall, after passing of this Act, continue to be the Constitution thereof; and that no alteration shall be made therein, unless by a vote to that effect of three-fourths of the resident members, and upon the request in writing of one-third of such resident members, and submitted at least one month before any vote shall be taken thereupon.

State of New York, Secretary's Office.

I CERTIFY the preceding to be a true copy of an original Act of the Legislature of this State, on file in this Office.

ARCH'D CAMPBELL,

ALBANY, April 29, 1818.

Dep. Sec'y.

ORDER OF COURT

ORDER OF THE SUPREME COURT OF THE STATE OF NEW YORK
TO CHANGE THE NAME OF
THE LYCEUM OF NATURAL HISTORY IN THE CITY
OF NEW YORK
TO
THE NEW YORK ACADEMY OF SCIENCES

WHEREAS, in pursuance of the vote and proceedings of this Corporation to change the corporate name thereof from "The Lyceum of Natural History in the City of New York" to "The New York Academy of Sciences," which vote and proceedings appear to record, an application has been made in behalf of said Corporation to the Supreme Court of the State of New York to legalize and authorize such change, according to the statute in such case provided, by Chittenden & Hubbard, acting as the attorneys of the Corporation, and the said Supreme Court, on the 5th day of January, 1876, made the following order upon such application in the premises, viz:

At a special term of the Supreme Court of the State of New York, held at the Chambers thereof, in the County Court House, in the City of New York, the 5th day of January, 1876:

Present—HON. GEO. C. BARRETT, *Justice*.

In the matter of the application of)
the Lyceum of Natural History
in the City of New York to au-
thorize it to assume the corporate
name of the New York Academy
of Sciences.

On reading and filing the petition of the Lyceum of Natural History in the City of New York, duly verified by John S. Newberry, the President and chief officer of said Corporation, to authorize it to assume the corporate name of the New York Academy of Sciences, duly setting forth the grounds of said application, and on reading and filing the affidavit of Geo. W. Quackenbush, showing that notice of such applica-

tion had been duly published for six weeks in the State paper, to wit, *The Albany Evening Journal*, and the affidavit of David S. Owen, showing that notice of such application has also been duly published in the proper newspaper of the County of New York, in which county said Corporation had its business office, to wit, in *The Daily Register*, by which it appears to my satisfaction that such notice has been so published, and on reading and filing the affidavits of Robert H. Browne and J. S. Newberry, thereunto annexed, by which it appears to my satisfaction that the application is made in pursuance of a resolution of the managers of said Corporation to that end named, and there appearing to me to be no reasonable objection to said Corporation to changing its name as prayed in said petition: Now on motion of Grosvenor S. Hubbard, of Counsel for Petitioner, it is

Ordered, That the Lyceum of Natural History in the City of New York be and is hereby authorized to assume the corporate name of The New York Academy of Sciences.

Indorsed: Filed January 5, 1876,

A copy.

WM. WALSH, *Clerk*.

Resolution of The Academy accepting the order of the Court, passed February 21, 1876

And whereas, The order hath been published as therein required, and all the proceedings necessary to carry out the same have been had, Therefore:

Resolved, That the foregoing order be and the same is hereby accepted and adopted by this Corporation, and that in conformity therewith the corporate name thereof, from and after the adoption of the vote and resolution herein above referred to, be and the same is hereby declared to be THE NEW YORK ACADEMY OF SCIENCES.

AMENDED CHARTER

MARCH 19, 1902

CHAPTER 181 OF THE LAWS OF 1902

AN ACT to amend chapter one hundred and ninety-seven of the laws of eighteen hundred and eighteen, entitled "An act to incorporate the Lyceum of Natural History in the City of New York," a Corporation now known as The New York Academy of Sciences and to extend the powers of said Corporation.

(Became a law March 19, 1902, with the approval of the Governor. Passed, three-fifths being present.)

The People of the State of New York, represented in Senate and Assembly, do enact as follows:

SECTION I. The Corporation incorporated by chapter one hundred and ninety-seven of the laws of eighteen hundred and eighteen, entitled "An act to incorporate the Lyceum of Natural History in the City of New York," and formerly known by that name, but now known as The New York Academy of Sciences through change of name pursuant to order made by the supreme court at the city and county of New York, on January fifth, eighteen hundred and seventy-six, is hereby authorized and empowered to raise money for, and to erect and maintain, a building in the city of New York for its use, and in which also at its option other scientific societies may be admitted and have their headquarters upon such terms as said Corporation may make with them, portions of which building may be also rented out by said Corporation for any lawful uses for the purposes of obtaining income for the maintenance of such building and for the promotion of the objects of the Corporation; to establish, own, equip, and administer a public library, and a museum having especial reference to scientific subjects; to publish communications, transactions, scientific works, and periodicals; to give scientific instruction by lectures or otherwise; to encourage the advancement of scientific research and discovery, by gifts of money, prizes, or other assistance thereto. The building, or rooms, of said Corporation in the City of New York used exclusively for library or scientific purposes shall be subject to the provisions and be entitled to the benefits of subdivision seven of section four of chapter nine hundred and eight of the laws of eighteen hundred and ninety-six, as amended.

SECTION II. The said Corporation shall from time to time forever hereafter have power to make, constitute, ordain, and establish such by-laws and regulations as it shall judge proper for the election of its officers; for prescribing their respective functions, and the mode of discharging the same; for the admission of new members; for the government of officers and members thereof; for collecting dues and contributions towards the funds thereof; for regulating the times and places of meeting of said Corporation; for suspending or expelling such members as shall neglect or refuse to comply with the by-laws or regulations, and for managing or directing the affairs or concerns of the said Corporation: and may from time to time alter or modify its constitution, by-laws, rules, and regulations.

SECTION III. The officers of the said Corporation shall consist of a

president and two or more vice-presidents, a corresponding secretary, a recording secretary, a treasurer, and such other officers as the Corporation may judge necessary; who shall be chosen in the manner and for the terms prescribed by the constitution of the said Corporation.

SECTION IV. The present constitution of the said Corporation shall, after the passage of this act, continue to be the constitution thereof until amended as herein provided. Such constitution as may be adopted by a vote of not less than three-quarters of such resident members and fellows of the said New York Academy of Sciences as shall be present at a meeting thereof, called by the Recording Secretary for that purpose, within forty days after the passage of this act, by written notice duly mailed, postage prepaid, and addressed to each fellow and resident member at least ten days before such meeting, at his last known place of residence, with street and number when known, which meeting shall be held within three months after the passage of this act, shall be thereafter the constitution of the said New York Academy of Sciences, subject to alteration or amendment in the manner provided by such constitution.

SECTION V. The said Corporation shall have power to consolidate, to unite, to co-operate, or to ally itself with any other society or association in the city of New York organized for the promotion of the knowledge or the study of any science, or of research therein, and for this purpose to receive, hold, and administer real and personal property for the uses of such consolidation, union, co-operation, or alliance subject to such terms and regulations as may be agreed upon with such associations or societies.

SECTION VI. This act shall take effect immediately.

STATE OF NEW YORK,

OFFICE OF THE SECRETARY OF STATE.

I have compared the preceding with the original law on file in this office, and do hereby certify that the same is a correct transcript therefrom, and the whole of said original law.

Given under my hand and the seal of office of the Secretary of State, at the city of Albany, this eighth day of April, in the year one thousand nine hundred and two.

JOHN T. McDONOUGH,
Secretary of State.

CONSTITUTION

ADOPTED, APRIL 24, 1902, AND AMENDED AT SUBSEQUENT TIMES

ARTICLE I. The name of this Corporation shall be The New York Academy of Sciences. Its object shall be the advancement and diffusion of scientific knowledge, and the center of its activities shall be in the City of New York.

ARTICLE II. The Academy shall consist of eight classes of members, namely: Sustaining Members, Active Members, Fellows, Associate Members, Student Members, Affiliated Members, Corresponding Members, and Honorary Members. Sustaining and Active Members shall be the members of the Corporation who live in or near the City of New York, or who, having removed to a distance, desire to retain their connection with the Academy. Fellows shall be chosen from the Sustaining and Active Members in virtue of their scientific attainments. Associate Members are members living at a distance from the City of New York, who wish to maintain a membership connection with the Academy without the responsibility and privileges of Active Membership. Student Members are junior workers in science connected with scientific institutions, as regular or research students, or junior faculty members, who wish temporary membership in the Academy. Corresponding and Honorary Members shall be chosen from among persons who have attained distinction in some branch of science. The number of Corresponding Members shall not exceed two hundred, and the number of Honorary Members shall not exceed fifty.

ARTICLE III. None but Fellows, Sustaining Members and Active Members who have paid their dues up to and including the last fiscal year shall be entitled to vote or to hold office in the Academy.

ARTICLE IV. The officers of the Academy shall be a President, as many Vice-Presidents as there are sections of the Academy, a Corresponding Secretary, a Recording Secretary, a Treasurer, a Librarian, an Editor, nine elected Councilors and one additional Councilor from each allied society or association. The annual election shall be held on the third Monday in December, or on some other day in December specifically designated by vote of the Council, the officers then chosen to take office at the first meeting in January following.

There shall also be elected at the same time a Finance Committee of three.

ARTICLE V. The officers named in Article IV shall constitute a council, which shall be the executive body of the Academy with general control over its affairs, including the power to fill *ad interim* any va-

cancies that may occur in the offices. Past Presidents of the Academy shall be *ex-officio* members of the Council.

ARTICLE VI. Societies organized for the study of any branch of science may become allied with The New York Academy of Sciences by consent of the Council. Members of allied societies may become Active Members of the Academy by paying the Academy's annual fee, but as members of an allied society they shall be Affiliated Members with the rights and privileges of Student Members, except the receipt of its publications. Each allied society shall have the right to delegate one of its members, who is also an Active Member of the Academy, to the Council of the Academy, and such delegate shall have all the rights and privileges of other Councilors.

ARTICLE VII. The President and Vice-Presidents shall not be eligible to more than one re-election; the Secretaries and Treasurer shall be eligible to re-election without limitation. The President, Vice-Presidents and Secretaries shall be Fellows. The terms of office of elected Councilors shall be three years, and these officers shall be so grouped that three, at least one of whom shall be a Fellow, shall be elected and three retired each year. Councilors shall not be eligible to re-election until after the expiration of one year.

ARTICLE VIII. The election of officers shall be by ballot, and the candidates having the greatest number of votes shall be declared duly elected.

ARTICLE IX. Ten members, the majority of whom shall be Fellows, shall form a quorum at any meeting of the Academy at which business is transacted.

ARTICLE X. The Academy shall establish by-laws, and may amend them from time to time as therein provided.

ARTICLE XI. This Constitution may be amended by a vote of not less than three-fourths of the Fellows and three-fourths of the Active Members present and voting at a regular business meeting of the Academy, provided that such amendment shall be publicly submitted in writing at the preceding business meeting, and provided also that the Recording Secretary shall send a notice of the proposed amendment at least ten days before the meeting, at which a vote shall be taken, to each Fellow and Active Member entitled to vote.

BY-LAWS

AS ADOPTED, OCTOBER 6, 1902, AND AMENDED AT SUBSEQUENT TIMES

CHAPTER I

OFFICERS

1. *President.* It shall be the duty of the President to preside at the business and special meetings of the Academy and also at the meetings of the Council.

2. *Vice-Presidents.* In the absence of the President, the senior Vice-President, in order of Fellowship, shall act as the presiding officer.

3. *Corresponding Secretary.* The Corresponding Secretary shall keep a corrected list of the Honorary and Corresponding Members, their titles and addresses, and shall conduct all correspondence appertaining to such membership. He shall make a report at the Annual Meeting.

4. *Recording Secretary.* The Recording Secretary shall keep the minutes of the meetings of the Academy and of the Council; he shall have charge of all records of the Academy, and of its corporate seal, which he shall affix and attest as directed by the Council; he shall keep a corrected list of the Active Members and Fellows, and shall send to them announcements of the Meetings of the Academy; he shall notify all Members and Fellows of their election, and members of committees of their appointment; he shall notify the members of the Council of the dates of the meetings thereof; he shall lay before the Council at each meeting all matters which have come to his attention since the last meeting and which require the consideration of the Council.

5. *Treasurer.* The Treasurer shall receive all membership fees and dues, all interest accruing and paid on the invested or other funds of the Academy, and contributions to the Treasury of the Academy from any other source. All such moneys, as received, shall be deposited in Banks or Trust Companies approved and designated by the Council as depositories, in the corporate name of the Academy and subject to the drafts of the Treasurer, as such. All bills and debts against the Academy shall be paid by the Treasurer on the order of the Council for their discharge.

The Treasurer shall report to the Council at each meeting thereof a statement of the current income and expenditures, and at the Annual Meeting, he shall report to the Academy the balance sheet of the funds and the income account for the preceding year.

6. *Librarian.* The Librarian shall have charge of the library, under the general direction of the Library Committee of the Council, and shall conduct all correspondence respecting exchanges of the Academy. He shall make a report on the condition of the library at the Annual Meeting.

7. *Editor.* The Editor shall have charge of the publications of the Academy, under the general direction of the Publication Committee of the Council. He shall make a report on the condition of the publications at the Annual Meeting.

CHAPTER II

COUNCIL

1. *Meetings.* The Council shall hold meetings at such stated times as it may decide upon, or at the call of the President. The Council shall have general charge of the affairs of the Academy.

2. *Quorum.* Five members of the Council shall constitute a quorum.

3. *Officers.* The President, Vice-Presidents and Recording Secretary of the Academy shall hold the same offices in the Council.

4. *Committees.* The Standing Committees of the Council shall be: (1) an Executive Committee consisting of the President, Treasurer, and Recording Secretary; (2) Such other committees as from time to time shall be authorized by the Council.

CHAPTER III

FINANCE COMMITTEE

The Finance Committee of the Academy shall audit the Annual Report of the Treasurer, and shall act with him in the recommendation to the Council of investments of funds of the Academy.

CHAPTER IV

ELECTIONS

1. *Sustaining, Active and Associate Members.* (a) These Members shall be nominated in writing to the Council by at least two Members who are qualified to do so by right of full membership privileges. If approved by the Council, they may be elected by the unanimous vote of the Councilors present at the meeting at which their names are presented.

(b) Any Active Member who, having removed to a distance from the city of New York, shall nevertheless express a desire to retain his connection with the Academy, may be placed by vote of the Council on the list of Associate Members. Such members shall relinquish the full privileges and obligations of Active Members, except the payment of an Associate Membership fee. They may receive the publications, in accordance with the recommendation of the council. (*Vide* Chapters V and X.)

2. *Student Members.* Junior workers in science, connected as students, junior research workers, or junior instructors with scientific institutions in or near New York City, may be elected to Student Membership for a period of five years in the manner prescribed for Active Members. They shall not have the power to vote and shall not be eligible to election as Fellows, but may receive the publications in accordance with the recommendations of the Council. At any time subsequent to their election they may assume the full privileges of Active Members by paying the dues of such Members.

3. *Fellows, Corresponding Members and Honorary Members.* Nominations for Fellows, Corresponding Members and Honorary Members may be made in writing either to the Recording Secretary or to the Council at its meeting prior to the Annual Meeting. If approved by the Council, the nominees shall then be balloted for at the Annual Meeting.

4. *Officers.* Nominations for Officers, with the exception of Vice-Presidents, may be sent in writing to the Recording Secretary, with the name of the proposer, at any time not less than thirty days before the Annual Meeting. Each section of the Academy shall nominate a candidate for Vice-President for the approval of the Council, who, on election as such, shall be Chairman of the section; the names of such nominees shall be sent to the Recording Secretary properly certified by the sectional secretaries, not less than thirty days before the Annual Meeting. The Council shall then prepare a list which shall be the regular ticket. This list shall be mailed to each Active Member and Fellow at least one week before the Annual Meeting. But any Active Member or Fellow entitled to vote shall be entitled to prepare and vote another ticket.

CHAPTER V

DUES

1. *Dues.* The annual dues of Sustaining Members shall be \$10, payable in advance at the time of the Annual Meeting.

The annual dues of Active Members shall be \$5, payable in advance at the time of the Annual Meeting.

The annual dues of Student Members shall be \$3, payable in advance at the time of the Annual Meeting.

Non-resident Associate Members shall pay an annual fee of \$3, so long as they are not entitled to the privileges of Active Membership. (*Vide* Chapter X.)

2. *Members in Arrears.* If any Member whose dues remain unpaid for more than one year, shall neglect or refuse to pay the same within three months after notification by the Treasurer, his name may be erased from the rolls by vote of the Council. Upon payment of his arrears, however, such person may be restored to Membership or Fellowship by vote of the Council.

3. *Renewal of Membership.* Any Active Member or Fellow who shall resign because of removal to a distance from the city of New York, or any Associate Member, may be restored by vote of the Council to Active Membership or Fellowship at any time upon application.

Associate Members not previously Active Members of the Academy may become Active Members, when approved by the Council, upon payment of the regular Active Membership fee.

CHAPTER VI

PATRONS, DONORS AND LIFE MEMBERS

1. *Patrons.* Any person contributing at one time \$1,000 to the general funds of the Academy shall be a Patron and, on election by the Council, shall enjoy all the privileges of an Active Member.

2. *Donors.* Any person contributing \$50 or more annually to the general funds of the Academy shall be termed a Donor and, on election by the Council, shall enjoy all the privileges of an Active Member.

3. *Life Members.* Any Member or Fellow contributing at one time \$100 to the general funds of the Academy shall be a Life Member and shall thereafter be exempt from annual dues; and any Sustaining Member who has paid annual dues for twenty-five years or more may, upon his written request, be made a Life Member and be exempt from further payment of dues.

CHAPTER VII

SECTIONS

1. *Sections.* Sections devoted to special branches of Science may be established or discontinued by the Academy on the recommendation

of the Council. The present sections of the Academy are the Section of Biology, the Section of Geology and Mineralogy, the Section of Anthropology, the Section of Psychology, the Section of Physics and Chemistry, and the Section of Oceanography and Meteorology.

2. *Organization.* Each section of the Academy shall have a Chairman and a Secretary, who shall have charge of the meetings of their Section. The regular election of the Secretary shall take place at the October or November meeting of the section and the Secretary-elect shall take office on the January first following. At the time of the election of the Secretary the section shall nominate a candidate for Vice-President of the Academy, who, if approved by the Council, on election as such, shall become Chairman of the section. Vacancies in these offices shall be filled pro-tempore by vote of the section; a chairman so elected being thereby nominated to the Council of the Academy for election as a Vice-President.

3. *Affiliation.* Members of scientific societies affiliated with the Academy, and members of the Scientific Alliance, or men of science introduced by members of the Academy, may attend the meetings and present papers under the general regulations of the Academy.

CHAPTER VIII

MEETINGS

1. *Business Meetings.* Business meetings of the Academy shall be held on the first Monday of each month from October to May inclusive.

2. *Sectional Meetings.* Sectional meetings shall be held on Monday evenings from October to May inclusive, and at such other times as the Council may determine. The sectional meeting shall follow the business meeting when both occur on the same evening.

3. *Annual Meeting.* The Annual Meeting shall be held on the third Monday in December, or on some other day in December specifically designated by vote of the Council.

4. *Special Meetings.* A special meeting may be called by the Council, provided one week's notice be sent to each Active Member and Fellow, stating the object of such meeting.

CHAPTER IX

ORDER OF BUSINESS

1. *Business Meetings.* The following shall be the order of procedure at business meetings:

1. Minutes of the previous business meeting.

2. Report of the Council.
3. Reports of Committees.
4. Elections.
5. Other business.

2. *Sectional Meetings.* The following shall be the order of procedure at sectional meetings:

1. Minutes of the preceding meeting of the section.
2. Presentation and discussion of papers.
3. Other scientific business.

3. *Annual Meetings.* The following shall be the order of procedure at Annual Meetings:

1. Annual reports of the Corresponding Secretary, Recording Secretary, Treasurer, Librarian, and Editor.
2. Election of Honorary Members, Corresponding Members, and Fellows.
3. Election of officers for the ensuing year.
4. Address of the retiring President.

CHAPTER X

PUBLICATIONS

1. *Publications.* The established publications of the Academy shall be the *Annals*, the *Special Publications*, the *Transactions*, the *Memoirs*, and *The Scientific Survey of Porto Rico and the Virgin Islands*. They shall be issued by the Editor under the supervision of the Committee on Publications.

2. *Distribution.* One copy of the established publications shall be sent to each Benefactor, Patron, Donor, Life Member, Sustaining Member, Active Member and Fellow.

3. *Publication Fund.* Contributions may be received for the publication fund, and the income thereof shall be applied toward defraying the expenses of the scientific publications of the Academy.

CHAPTER XI

GENERAL PROVISIONS

1. *Debts.* No debts shall be incurred on behalf of the Academy, unless authorized by the Council.

2. *Bills.* All bills submitted to the Council must be certified as to correctness by the officers incurring them.

3. *Investments.* Funds in hand, arising from the maturity of existing investments or from surplus income, shall be invested by the Council in first mortgage loans or real estate or in approved negotiable securities recommended by the Finance Committee in joint action with the Treasurer.

4. *Permanent Fund.* Contributions and fees received from Benefactors, Donors and Life Members shall be credited to the Permanent Fund.

5. *Expulsion, etc.* Any Member or Fellow may be censured, suspended or expelled for violation of the Constitution or By-Laws, or for any offence deemed sufficient, by a vote of three-fourths of the Members and three-fourths of the Fellows present at any business meeting, provided such action shall have been recommended by the Council at a previous business meeting, and also, that one month's notice of such recommendation and of the offence charged shall have been given the Member accused.

6. *Changes in By-Laws.* No alteration shall be made in these By-Laws unless it shall have been submitted publicly in writing at a business meeting, shall have been entered on the Minutes with the names of the Members or Fellows proposing it, and shall be adopted by two-thirds of the Members and Fellows present and voting at a subsequent business meeting.

MEMBERSHIP
OF
THE NEW YORK ACADEMY OF SCIENCES
AUGUST 1, 1944
HONORARY MEMBERS

lected

- 1943 O. T. AVERY, New York, N. Y.
- 1889 CHARLES EUGENE BARROIS, Lille, France
- 1937 ORPEN BOWER, Glasgow, Scotland
- 1936 HENRI BREUIL, Paris, France
- 1935 ROBERT BROOM, Pretoria, South Africa
- 1936 MAURICE CAULLERY, Paris, France
- 1937 PIERRE TEILHARD DE CHARDIN, Peiping, China
- 1942 ARTHUR H. COMPTON, Chicago, Illinois
- 1938 EDWIN GRANT CONKLIN, Philadelphia, Pennsylvania
- 1935 CLIVE FORSTER COOPER, London, England.
- 1942 CLINTON J. DAVISSON, New York, N. Y.
- 1941 PETER J. W. DEBYE, Ithaca, New York
- 1920 GERHARDT DE GEER, Stockholm, Sweden
- 1936 OCTAVE DUBOSQ, Paris, France
- 1943 ALEXANDER FLÉMING, London, England
- 1935 S. A. FREDERICKSSON, Stockholm, Sweden
- 1935 EDWIN STEPHEN GOODRICH, Oxford, England
- 1938 ROSS GRANVILLE HARRISON, New Haven, Connecticut
- 1943 FREDERICK GOWLAND HOPKINS, Cambridge, England
- 1935 LELAND O. HOWARD, Washington, D. C.
- 1936 JULIAN S. HUXLEY, London, England
- 1940 ARTHUR KEITH, Kent, England
- 1943 ALFRED I. KROEBER, Berkeley, California
- 1909 ANTOINE FRANCOIS ALFRED JACROIX, Paris, France
- 1938 IRVING LANGMUIR, Schenectady, New York.
- 1935 H. LUNDBORG, Stockholm, Sweden
- 1936 KARL SPENCER LANHILEY, Orange Park, Florida
- 1935 JOHN CAMPBELL MERRIAM, Pasadena, California
- 1935 PETER CHALMERS MITCHELL, London, England
- 1936 KINGO MIYABE, Sapporo, Japan.
- 1942 THOMAS H. MORGAN, Pasadena, California
- 1937 D. OBRUGEV, Moscow, Russia
- 1937 CHARLES PALACHE, Cambridge, Massachusetts
- 1935 GEORGE HOWARD PARKER, Cambridge, Massachusetts
- 1898 FRIEDRICH CARL ALBRECHT PENCK, Berlin, Germany
- 1936 CHARLES PEREZ, Paris, France
- 1911 EDWARD BAGNALL POULTON, Oxford, England
- 1913 DAVID PRAIN, Surrey, England
- 1939 HENRY NORRIS RUSSELL, Princeton, New Jersey
- 1935 WILLIAM BERRYMAN SCOTT, Princeton, New Jersey

- 1937 H. SPEMANN, Freiburg, Germany
 1935 ERIK A. STENSIÖ, Stockholm, Sweden
 1943 TH. SVEDBERG, Uppsala, Sweden
 1942 HARALD U. SVERDRUP, La Jolla, California
 1943 ARNE TISELIUS, Uppsala, Sweden
 1935 DAVID MEREDITH SEARES WATSON, London, England
 1937 FRANZ WEIDENREICH, New York, N. Y.
 1935 ARTHUR SMITH WOODWARD, Sussex, England

ACTIVE MEMBERS

Fellowship is indicated by an asterisk (*) before the name

PATRON

Roebling, John A.

LIFE MEMBERS

- *Allis, Edward Phelps, Jr., LL.D., M.D.
 *Andrews, Roy C., Sc.D.
 *Anthony, Harold E., Sc.D.
 *Armstrong, Clairette P., Ph.D.
 *Armstrong, S. T., Ph.D., M.D.
 Arnold, Weld, A.B.
 *Ashby, George E.
 Auchincloss, Hugh D.
 *Barnhart, John H., M.D.
 Beecher, Henry K., M.D.
 *Berkey, Charles Peter, Ph.D., Sc.D.
 Berthelsdorf, Siegfried, M.D.
 Billings, Elizabeth
 *Bird, Henry
 Bird, Whitworth F.
 *Bliss, Charles B., Ph.D., B.D.
 *Burdick, C. Lalor, Ph.D.
 *Byrnes, Esther F., Ph.D.
 *Chapman, Frank M., Sc.D.
 Clark, F. Ambrose
 Cochran, Henry J., A.B.
 *Cooke, C. Montague, Jr., Ph.D.
 *Crampton, Henry E., Ph.D., Sc.D.
 Crunden, Allan B., Jr., M.D.
 *Curtis, Carlton C., Ph.D.
 *Davis, William T.
 *Dodge, Richard E., A.M.
 *Dubos, Rene J., Ph.D.
 *Dunn, Gano, E. E., Sc.D.
 Dunscombe, George Elsworth
 Ellis, Ralph
 *Elwyn, Adolph, A.M.

- Evans, Florence L., Ph.D.
 *Falk, K. George, Ph.D.
 Field, W. B. Osgood
 *Finlay, George I., Ph.D.
 *Fisher, G. Clyde, Ph.D., LL.D.
 *Frick, Childs
 Gallatin, Albert
 *Grabau, Amadeus W., Ph.D.
 *Gregory, William K., Ph.D., Sc.D.
 *Haskins, Caryl P., Ph.D.
 *Hay, Clarence L., A.M.
 *Hildburgh, Walter L., Ph.D.
 *Hubbard, Bela, Ph.D.
 *Huntington, Archer M., Litt.D., LL.D.
 *Hussakof, Louis, Ph.D.
 Hyde, James H., Litt.D.
 Iddings, Carl, Ph.D.
 *Kovarik, Alois F., Ph.D., Sc.D.
 *Lieb, Charles C., M.D.
 Lindboe, S. R.
 *McCulloch, Warren Sturgis, M.D.
 McKim, Orville Ernest, D.V.S.
 *Meyerhoff, Howard A., Ph.D.
 *Miner, Roy Waldo, Ph.D., Sc.D.
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 Morgan, William Fellowes, F.M.
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 Newcomer, H. Sidney, M.D.
 *Notman, Howard
 Notman, Katharine H.
 Oakes, Byron J., Ph.D.
 *O'Connell, Daniel T., M.S., Sc.M.,
 Ph.D.
 *Ogilvie, Ida H., Ph.D.
 Osborn, Mrs. William C.

- *Osburn, Raymond C., Ph.D.
- *Pike, Frank H., Ph.D.
Plaut, Edward, Ph.D.
Porro, Thomas J.
- *Prince, John D., Ph.D.
Quigley, Leon V., M.S.
- *Reeds, Chester A., Ph.D.
Rice, Hugh S., A.M.
Roger, John
- *Shapiro, Harry L., Ph.D.
Sloan, Benson Bennett, B.A.
- *Stefansson, Vilhjalmur, LL.D., Ph.D.
- *Taylor, Norman
Ward, Charles Willis
Warner, Mrs. H. W.
Webb, Addison, B.A.
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White, Amelia E.
Wiley, Robert M.
- *Wilson, Margaret B., M.D.
- *Wissler, Clark, Ph.D., LL.D.
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- *Woodworth, R.S., Ph.D., Sc.D., LL.D.
- Ciampolini, Ettore, M.D., C.P.H.
Ph.D.
Clark, E. Mabel
- *Clarke, Hans T., D.Sc.
Clayburgh, A.
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- *CoTui, Frank, M.D.
- *Cowles, Edward Spencer, M.D.
Coykendal, Frederick, C.E., A.M.,
LL.D.
- *Dahlgren, B. E., M.D.
- *Davison, F. Trubee, LL.D.
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- *Dunning, William Bailey, D.D.S.
Eising, Eugene H., M.D.
- *Eno, William Phelps, M.A.
- *Eppley, Marion, Ph.D.
Feinstein, Robert R.
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Garcia-Cabrera, Esteban, M.D.
- *Greenwald, Isidor, Ph.D.
- Gregory, Angela DuBois
- *Griscom, Ludlow, A.M.
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- *Halsey, Robert H., M.D.
- *Harper, R. A., Ph.D., Sc.D.
- *Hauser, Ernst A., Ph.D.
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- Holbein, William G.
- *Holden, Francis, Ph.D.
Hunter, Fenley
- *Iselin, Columbus O'D., M.A.
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Joyner, A. L., B.Sc., M.D.
- Kaufmann, Jacob, M.D.
- *Kern, Edward F., Ph.D.
- *Kerr, Paul F., Ph.D.
Kollmorgen, Frederick L.
Kosch, Irving G.
- *Krasnow, Frances, Ph.D.
- Lawton, Shailer U., M.D.
- *Lorge, Irving, Ph.D.
McEwen, Alfred
- *MacInnes, Duncan A., Ph.D.

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- *Ames, Oakes, A.M.
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- *Assmuth, Joseph, Ph.D.
- *Auchincloss, Hugh, M.D.
Barzilai, Gemma, M.D.
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Betts, Wyllys Rosseter, Jr.
- *Blair, W. Reid, D.V.S., LL.D.
- *Breder, Charles M., Jr., D.Sc.
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- *Brill, A. A., M.D.
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- *Brown, Barnum, Sc.D.
- *Brown, Samuel A., M.D.
- *Burrow, Trigrant, M.D., Ph.D.
Carabba, Victor, M.D., D.V.S.
- *Carhart, Grace M., A.M.
- Carlisle, James M., M.D.
- Chesley, Leon C., Ph.D.
- *Childs, George Henshaw, Ph.D.

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 *Miner, Eunice Thomas
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 *Morrison, A. Cressy, M.R.I.
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 *Plaut, Alfred, M.D.
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 Pyle, Norman J., V.M.D.
 *Rautenstrauch, Walter, LL.D.
 Rice, Otis R., B.A., B.D.
 *Robbins, William J., Ph.D., Sc.D.
 Robinson, Cortland O., M.D.
 Roth, Harry, B.S.
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 *Schaefer, Hugo H., Ph.D., Ph.D.
 *Schwarz, Herbert F., M.A.
 *Semken, George H., M.D.
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 Smith, Ismael
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 *Stunkard, Horace W., Ph.D., Sc.D.
 Sweet, William Otis, B.S.
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 Thorn, Marvin D., B.S.
 Thorne, Samuel, LL.B.
 Tibbals, Samuel G.
 Walker, Miriam Dwight
 Wallerstein, Leo
 *Weber, Orlando F.
 *Weiss, Harry B.
 *Wellnitz, Anna M., Ph.D.

*Whitlock, Herbert P., C.E.

*Zachry, Caroline B., Ph.D.
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ACTIVE MEMBERS

Abbott, Theodore J., M.D.
 Abel, Theodora Mead, Ph.D.
 *Abramson, Harold A., M.D.
 Abramson, Sylvia, A.B.
 Aceonci, Themistocles F. D., M.A.
 Ackermann, Alfred J., M.D.
 Ackermann, Edward A., Chem.E.
 Ackermann, Wolfgang, M.D.
 Adams, Mark H., Ph.D.
 Adams, Mildred, Ph.D.
 Akin, Russell B., Ph.D.
 *Albee, Fred H., M.D., Sc.D., LL.D.
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 Allen, Alexander A., M.A., D.D.S.
 *Alling, Harold L., Ph.D.
 *Anastasi, Anne, Ph.D.
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 Anson, M. L., Ph.D.
 Antopol, William, M.D.
 Applebaum, Edmund, D.D.S.
 Appleby, Alfred N., Ph.D.
 Archbold, Richard
 Archibald, Reginald M., Ph.D., M.D.
 Arensberg, Conrad M., Ph.D.
 Armstrong, Elizabeth J., Ph.D.
 Armstrong, George S., M.E., C.E.
 *Arthur, John M., Ph.D.
 Asch, Solomon E., Ph.D.
 Atherley, Leonard F., B.S.
 Atz, James W., A.B.
 Auchincloss, Reginald, Ph.D.
 Auerbach, Johann G., M.D.
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 Babor, Joseph A., Ph.D.
 *Bachman, George W., Ph.D.
 Bailey, John H., Ph.D., D.P.H.

- *Bailey, Percy Laurence, Jr., Ph.D.
- Bainbridge, William C., B.S.
- Baker, Ross A., Ph.D., Sc.D.
- Bakhmeteff, Boris A., D.Eng.
- Bakst, Aaron, Ph.D.
- Bakst, James, Ph.D.
- Balce, Sofronio, M.S.
- Ball, C. Olin, Ph.D.
- Ballintine, Robert, Ph.D.
- Ballou, Charles H., B.S.
- Baltazar, Eulalio P.
- Banay, Ralph S., M.D.
- Barnes, Marion David, Ph.D.
- Barnes, R. Bowling, Ph.D.
- Barnes, Robert P., Ph.D.
- Baron, Harry, M.S.
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- Bateman, John B., Ph.D.
- *Bateson, Gregory, M.A.
- *Beach, Frank A., Ph.D.
- Beale, Helen Purdy, Ph.D.
- Beam, Rachael, A.M.
- Beard, Stanley D., Ph.B.
- Beck, Alfred D., M.A.
- Becker, Marie F.
- *Beckman, Charles O., Ph.D.
- Beckwith, Edward P., S.B.
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- *Bergmann, Max, Ph.D.
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- Berman, Louis, M.D.
- Bernard, Pierre A.
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- *Blain, Alexander W., M.D.
- *Blanchard, Kenneth C., Ph.D.
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- Blount, Raymond F., Ph.D.
- *Bodansky, Aaron, Ph.D.
- *Bodansky, Oscar, Ph.D., M.D.
- *Bodecker, Charles F., D.D.S.
- *Bogert, Charles M., M.A.
- Booth, Verne H.
- Bosce, Roland A., Ch.D.
- *Bowen, Earl, Ph.D.
- Boyd, William C., Ph.D.
- *Boyden, Alan A., Ph.D.
- Brackmier, Gladys H., A.M.
- Braestrup, Carl B., B.S.
- Bram, Joseph, Ph.D.
- Brand, Erwin, Ph.D.
- Brescia, Frank, Ph.D.
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- Brough, Glen A., B.Sc., M.D.
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- *Bucher, Walter H., Ph.D.
- Buell, C. Eugene, Ph.D.
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- Bunney, William E., Ph.D.
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- Burns, Dean C., M.D.
- *Burns, R. M., Ph.D.
- *Burr, Emily Thorp, Ph.D.
- Burstein, Elias, A.M.
- Burt C. Pauline, Ph.D.
- Burton, Milton, Ph.D.

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 *Butler, Bertram T., Ph.D.
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 *Caldwell, Mary L., Ph.D.
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 Campbell, H. Louise, Ph.D.
 Canfield, Roy H., M.F.
 Cann, Jessie Y., Ph.D.
 *Cannan, R. Keith, Sc.D.
 Cannon, A. Benson, M.D.
 Carey, Benjamin W., M.D.
 Carey, Eben, M.D.
 Carlson, Gustaf H., Ph.D.
 Cassidy, Harold G., Ph.D.
 Catalani, Arthur P., Ph.G., B.S.
 *Cattell, McKeen, Ph.D., M.D.
 *Cattell, Ware
 Cerecedo, L. R., Ph.D.
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 *Chambers, Leslie A., Ph.D.
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 *Chapin, James P., Ph.D.
 Chapman, George H.
 Charbonnel, Ernest A., D.D.S.
 Charipper, Harry A., Ph.D.
 Chase, Aurin M., Ph.D.
 *Cheney, Ralph H., Sc.D.
 Chenoweth, Maynard Burton, M.D.
 Chesire, Leone E., M.Sc.
 Chesley, Frank G., M.Sc.
 Chmielowiec, Steven A.
 Choucroun, Nine, D.Sc.
 *Chow, Bacon F., Ph.D.
 Christensen, L. Royal, Ph.D.
 Christian, Walter V., Ph.D.
 Chrystall, Frieda Lichtman, M.S.
 Clapp, Frances L., B.A.
 Clapp, Philip G., Ph.D.
 Clark, Allan C.
 Clark, Everett H.
 Clark, F. H., Sc.D.
 *Clark, Herbert C., M.D.
 Clark, LeRoy V., B.Sc.
 Clarke, Walter V., M.A.
 Clasby, Edward J.
 Claude, Albert, M.D.
 Clausen, Lucy W., B.S.
 Clement, Anthony C., Ph.D.
 Clemente, Amando, Ph.D.
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 Cohen, E. K.
 Cohen, M. U., Ph.D.
 *Cohn, Alfred E., M.D.
 *Cohn, Edwin J., Ph.D.
 *Colbert, Edwin H., Ph.D.
 *Cole, Kenneth S., Ph.D.
 Coleman, Bernard Simpson, S.B.
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 Colien, Francis E., Ph.D.
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 Collins, Donald L., Ph.D.
 Collins, Robert E. Lee, Ph.D.
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 Comstock, W. P., A.B.
 Conner, Robert T., Ph.D.
 *Connolly, Cornelius J., Ph.D.
 *Conrad, G. Miles, B.A.
 Conroy, Peter J., Ph.G., Ph.D.
 Conway, Hertha C.
 Cook, Charles A., M.S., Ph.D.
 Coons, Callie M., Ph.D.
 Coons, Robert Roy, Ph.D.
 Cooper, Edward B., M.A.
 Cooper, Stewart R., Ph.D.
 Cope, Otis M., M.D.
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 Cowles, Raymond B., Ph.D.
 *Cowley, William H., Ph.D., LL.D.
 Cox, Edward H., D.Sc.
 Craig, Gerald S., Ph.D.
 Craig, Gladys M., M.Sc.
 *Craig, Lyman C., Ph.D.
 *Crandall, Lee S.
 Crane, E. J., D.Sc.
 Creighton, H. Jermain, D.Sc.
 Crosman, Arthur M., Ph.D.
 *Crossley, M. L., Ph.D.
 Curphey, Theodore J., C.M., M.D.
 D'Alcilio, Gaetano F., Ph.D.

- Daniel, Janet, Ph.D.
 Dante, John Henry, M. S.
 Darken, L. S., Ph.D.
 Darkenwald, Gordon G., Ph.D.
 Darling, Hugo, M.D., Ph.D., E.F.
 *Darlington, Charles G., M.D.
 Darrow, Karl K., Ph.D.
 David, Pedro A., Ph.D.
 Davidheiser, Lee Y., Ph.D.
 *Davidson, David, Ph.D.
 Davidson, Morris, M.D.
 Davis, Bernard D., M.D.
 Davis, Herbert L., Ph.D.
 *Dawson, J. A., Ph.D.
 Dearing, A. Willis, Ph.D.
 deBodo, Richard Charles, M.D.
 de Carvajal-Foreto, J., Ph. B.
 DeGara, Paul F., M.D.
 *Degering, E. F., Ph.D.
 Dekker, Maurits, Dr.S.
 deLeon, Benjamin, M.A.
 Delchamps, H. J., B.E.
 DeSmitt, Vladimir P.
 Deutsch, Felix, M.D.
 Dewey, Jane Mary, Ph.D.
 Diamond, Moses, D.D.S.
 Diasio, F. A., M.D.
 DiCyan, Erwin, Ph.D.
 Dietz, Thomas J., M.S.
 Dingle, John H., M.D., Sc.D.
 Dingwall, Andrew, A.R.T.C., Ph.D.
 *Dodge, Bernard O., Ph.D.
 Doebbeling, Susie E., Ph.D.
 Dolan, Francis E., Sc.D.
 Dolley, William L., Ph.D.
 Donahue, James Kenneth, Ph.D.
 Doty, Paul Mead, Jr., Ph.D.
 Doubilet, Henry, M.D., M.Sc.
 Downes, Harold C., Ph.D.
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NOVEMBER 10, 1944

ENERGY RELATIONSHIPS IN ENZYME REACTIONS*

By

JOSEPH S. FRUTON, ERIC G. BALL, MAX BERGMANN, HERMAN M.
KALCKAR, OTTO MEYERHOF, AND CARL V. SMYTHE

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* This series of papers is the result of a conference on Energy Relationships in Enzyme Reactions held by the Section of Physics and Chemistry of The New York Academy of Sciences, February 11 and 12, 1944.

Publication made possible through a grant from the income of the Conference Publications Revolving Fund.

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INTRODUCTORY REMARKS

By

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This discussion on "Energy Relationships in Enzyme Reactions" is another in the series of conferences sponsored by the Section of Physics and Chemistry of the New York Academy of Sciences. The subject of our conference is essentially biochemical, but like all borderline disciplines, biochemistry owes much of its vitality to the nourishment it constantly derives from the older sciences from which it arose. It is appropriate, therefore, that we should welcome to this conference not only confessed biochemists, but also biologists, physiologists, organic chemists, physical chemists and physicists.

At this conference we propose to discuss the energetics of chemical reactions catalyzed by enzymes. The number of enzyme systems which have been investigated carefully from this point of view is small compared to the job still ahead. Nevertheless, enough information has accumulated regarding the chemical dynamics and energetics in biological systems to encourage the belief that the continued exploration of this field will give us a clearer picture of the chemical events in living cells.

For the biochemist, the prerequisites for the occurrence of a given chemical reaction are the same for a biological system as are those for a purely chemical system. Once the chemical structure of the participants in a reaction are known, the further study involves a determination of the factors which influence the equilibrium condition of the reaction and of the factors which affect the speed with which equilibrium is attained. The position of the equilibrium is a function of the energetics of the reaction and thermodynamic theory states that the reaction will approach equilibrium spontaneously only when the process involves a decrease in the free energy of the system. The importance of the knowledge of the sign and magnitude of the free energy change in biochemical reactions needs no emphasis at this point, since this question will figure importantly in the papers to be presented today and tomorrow.

What distinguishes biochemical reactions from chemical reactions in general is the means provided for the rapid attainment of equilibrium. Biological systems are uniquely characterized by the fact that they are equipped with highly specific catalysts, the enzymes. Although, on a thermodynamic basis, a very large number of reactions may be theoretically possible in a given biological system, the presence of enzymes

will result in the selective catalysis of only a limited number of these reactions. This enzymatic catalysis of selected chemical reactions thus determines the course of metabolic processes. It is clear, therefore, that in order to elucidate the course of chemical reactions in a biological system, it is essential to recognize the enzymes that are active in that system.

Much of the progress in our knowledge regarding the chemical events in living systems has been due to the advances made in the isolation and purification of enzymes. The impetus for this development came from the successful crystallization of urease by Sumner and of several of the proteolytic enzymes by Northrop and his associates. To date, about twenty enzymes have been obtained in crystalline form; the most recent success has been the crystallization of muscle phosphorylase by Green, Cori and Cori. In addition, several enzymes which have hitherto resisted crystallization have been obtained in a high degree of purity. The contributions of Warburg are perhaps the outstanding example of the way in which the availability of purified enzymes makes possible a clearer understanding of biochemical reactions. It is becoming increasingly apparent, therefore, that future advances in our knowledge of the chemical dynamics of living matter depend, in the first place, on the isolation, purification and study of the catalytic action of individual enzymes.

One important conclusion that has emerged from the studies on purified enzymes is that the catalytic activity was found, in each case, to be associated with a protein. The idea of the protein nature of enzymes, proposed by Traube in 1860 and restated by Emil Fischer in 1907, was widely accepted by biochemists until the prestige of the Willstätter school swayed opinion the other way. In the face of the work with crystalline enzymes, we have now returned to the view that enzymes are proteins.

In one sense, this is unfortunate, since it means that the complete clarification of the chemical constitution of enzymes depends on the solution of the problem of the structure of proteins. It looks as though we shall have to await further progress in the field of protein chemistry before we shall be able to discern those structural features that distinguish the enzymes from catalytically inactive proteins.

In another respect, however, what we do know about the chemical composition of proteins gives a clue to the basis for the extreme differences in specificity between enzymes. It seems probable that the precise specificity of each enzyme is the expression of a particular chemical structure, perhaps a particular arrangement of amino acid residues in

the enzyme molecule. Certainly, of the known constituents of living matter, there are few which are capable of the variations in chemical structure that are possible for proteins.

The structural elements of an enzyme protein that are responsible for its catalytic power are unknown. The assumption is made that there is an active catalytic center and that the first step in catalysis involves the combination of the substrate with the active center of the enzyme to form a dissociable enzyme-substrate complex. According to this view, during the period of its contact with the enzyme, the substrate is activated and this activated substrate molecule then undergoes rapid reaction with another substance. The reality of the enzyme-substrate combination, a concept clearly developed by Michaelis in 1913, is unquestioned today. Recent studies by Keilin, Stern, and Chance on catalase and peroxidase have provided striking additional evidence for the existence of enzyme-substrate compounds.

Mention of the enzymes catalase and peroxidase recalls the fact that each of them represents a complex protein in which a so-called prosthetic group, in this case a hemin, is linked to a specific protein. Both the catalytic activity and the specificity of action depend on the nature of this protein. The term prosthetic group has also been used to designate molecules such as the pyridine nucleotides and the alloxazine nucleotides which combine reversibly with specific proteins and, as a result, undergo rapid oxidation-reduction reactions. The brilliant researches on the chemical nature of these low molecular weight substances have perhaps tended to obscure the fact that the essential catalytic activity resides in the protein to which they become attached. The so-called prosthetic group is usually a partner in the chemical reaction catalyzed by the enzyme. The active catalytic center is represented by structural elements in the protein with which the prosthetic group combines.

Since we do not know the chemical nature of the active centers of enzymes, it is impossible today to visualize the mechanism of activation of the substrate in terms of the chemical structure of intermediate compounds, as has been possible in certain cases of catalysis by simple organic compounds. Langenbeck, for example, has found that certain simple organic substances can catalyze chemical reactions for which enzyme catalysts were known. This empirical approach is of considerable interest, but the identity, or even the similarity, of the active group in the model catalyst and in the enzyme still remains to be demonstrated.

Recent years have seen several developments in the application of the modern theories of chemical kinetics to the problems of enzyme catalysis. The view has been put forward by Haber and Willstätter and later by Moelwyn-Hughes and Waters that enzymes act as initiators of chain reactions. In addition, Eyring, Stearn and Medwedew have approached the question from the point of view of quantum chemistry. It is clear that future progress in our understanding of the mechanism of enzyme action depends greatly on the efforts of physical chemists to establish a consistent theory of chemical catalysis.

Pending these attempts to define the mechanism of enzyme action in the language of physics and chemistry, the biochemist will probably continue to explore the role of enzymes in biological systems. In particular, he will seek to discover the nature of the enzyme catalyzed chemical reactions that occur in such systems. The prominent reactions in the cell are the processes of hydrolysis and condensation and of oxidation-reduction. In addition to these major reactions, organic substances may undergo a variety of other reactions such as amination and deamination, alkylation and so forth. In the cell, many of these reactions are going on continuously and simultaneously. Large molecules such as the proteins are constantly being broken down into their constituents. These degradative processes are balanced by synthetic reactions. Since, in many cases, these synthetic reactions require energy for their occurrence, they must be coupled with other processes which will provide the driving force for synthesis. The steady state which is established represents, therefore, a dynamic equilibrium in which enzymes are continuously catalyzing not only degradation and regeneration reactions but also those chemical reactions which make available, for synthetic processes, the energy obtained from the environment. It is characteristic of the steady state in living matter that many of the individual chemical systems do not attain thermodynamic equilibrium, but are poised at concentration ratios different from those expected on the basis of thermodynamic data. Thus, the position of the steady state is determined, not only by the thermodynamic potentials, but also by the kinetics of the individual enzyme catalyzed reactions.

These conferences were inaugurated a number of years ago in order to provide an opportunity for workers in actively developing fields to meet and to discuss their problems. The active development of the subject of the present conference is unquestioned. It may not be too much to hope that this discussion concerning the energetics of biochemical catalysis will in itself serve as a catalyst for future research

ENERGY RELATIONSHIPS OF THE OXIDATIVE ENZYMES

BY ERIC G. BALL

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Life, from the viewpoint of the physical chemist, may be defined as a manifestation of the transformation of energy by the living organism. The plant, for example, through the agency of chlorophyll, is able to transform solar energy into potential chemical energy by the synthesis from CO_2 and water of carbohydrates, fats, and proteins. The animal, on the other hand, is able to release this stored solar energy by converting these plant products back again into CO_2 and water. It is, of course, by this procedure, which we term metabolism, that the animal obtains the energy by which he moves himself and surrounding objects, by which he maintains a body temperature usually higher than his environment, and by which he performs a multitude of processes whose study is the delight of the physiologist and which constitute life.

During the past twenty years, our knowledge of the metabolic processes whereby carbohydrates, fats, and proteins undergo degradation in the animal body has increased greatly. To be sure, we are still far from a complete understanding of the processes involved, but we have advanced far enough so that probing fingers are already beginning to search for the mechanism whereby this energy release is geared to perform the functions essential to life. Let us, therefore, consider briefly what we know today concerning the oxidative reactions by which foodstuffs are converted into CO_2 and water and see what deductions we may draw concerning the amount of energy thereby released and its utilization by the living cell.

In the consideration of the energy release of foodstuffs during oxidation, it has been the custom to write an over-all equation for the reaction such as the one shown here for glucose:



In this process of combustion, we have learned that whether it takes place in the body or *in vitro*, the same amount of heat, ΔH , is evolved, and this is 673,000 calories for each gram molecular weight of glucose burned. Now, the expression of energy release as heat in this equation, though undoubtedly satisfactory for the purpose of calculating food re-

quirements of an individual, is not suitable if we wish to discuss accurately the energy relationships of the oxidative processes in the body. Animals are not heat engines and as such can utilize only the free energy, ΔF , of such reactions to carry out their various physiological functions. It is, thus, with ΔF that we must deal in our considerations of the energy of a system which is convertible into useful work in the animal body. For an isothermal process, the two quantities ΔF and ΔH are related to one another by the fundamental thermodynamic equation

$$\Delta F = \Delta H - T\Delta S \quad (2)$$

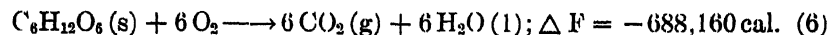
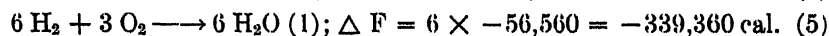
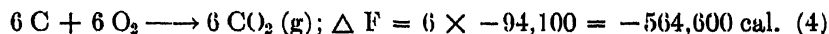
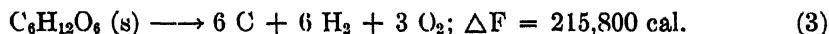
where T is the absolute temperature and ΔS is the entropy of the system under consideration.

There are four general methods which have been employed in determining the value of the free energy change, ΔF , in various chemical reactions. As stated by Parks and Huffman⁹ these are:

1. The measurement of the equilibrium constant, K , in a "reversible" reaction and the calculation of ΔF by the relation $\Delta F = -RT \ln K$.
2. The determination of the reversible electromotive force, E , of an electrical cell involving the reaction in question, in which case $\Delta F = -NFE$.
3. The determination of heat capacities down to very low temperatures and the utilization of these results with other thermal data to calculate ΔF by means of the third law of thermodynamics.
4. The combination of suitable chemical equations with known ΔF values to give the reaction in question.

We shall have occasion to use ΔF values calculated by all four methods in the discussion that follows.

Let us now take a typical foodstuff, glucose, and calculate as follows the free energy for the reaction in which it undergoes combustion:



The values employed in equations (3), (4), and (5) are taken from Parks and Huffman.⁹ The summation of these equations yields equation (6) and a value for $\Delta F = -688,160$ calories. There are thus released 688,160 calories capable of performing work when one gram molecule of glucose undergoes oxidation to CO_2 and H_2O . It should be noted that though ΔF is nearly equal to ΔH for this reaction (equation 1), this is not true for all reactions. For example, in the conversion of glucose to lactate, ΔF is approximately 50 per cent greater than ΔH .

Now equation (6) tells us nothing concerning the mode of oxidation of glucose in the animal body. We know, however, from the first law of thermodynamics that regardless of the pathway by which this oxidation occurs, the same number of calories will be released as free energy. Actually, however, the amount of free energy released in the combustion of glucose in the animal body will not be exactly equal to that stated in equation (6). This is because the equation as written applies only when all substances participating in it are at the standard state and the reaction occurs at 25° C. To obtain the corresponding free energy for the process as it occurs in the mammalian body, corrections should, of course, be made so that all the participants are at the concentration met with in the body and at a temperature of 38° C. Since these corrections would, however, amount to but a small fraction of the total free energy, they will be ignored for the sake of simplicity in most of the discussion that follows.

Now, before we can proceed further with our consideration of energy relationships, it is necessary for us to inquire into what is known concerning the mechanism of biological oxidations in the animal body. We may schematize the chief process by which foodstuffs appear to be oxidized in the living cell in the manner shown in FIGURE 1. The food-

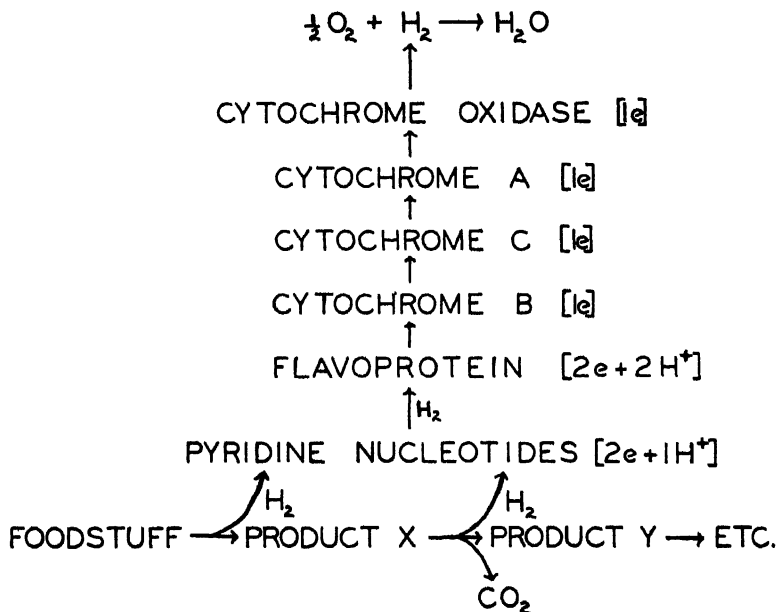


FIGURE 1

stuff, on entering the cell, undergoes an enzymatic reaction in which two hydrogen ions and two electrons (or two hydrogen atoms) are removed and in which one of the pyridine nucleotides is reduced. There then follows a series of oxidations and reductions involving a flavoprotein, the three cytochromes, and cytochrome oxidase, by which the pyridine nucleotide is reoxidized and the hydrogen ions and electrons are transferred to oxygen with the formation of water. The dehydrogenated product (X) of the foodstuff then undergoes a similar enzymatic reaction in which a different enzyme from the first one is required, though the same pyridine nucleotide may again act as coenzyme. Two hydrogens are again removed and their transfer to oxygen then follows the same pathway as the previous two. The breakdown of the foodstuff thus continues to completion, step by step, with the liberation of two hydrogen atoms at each step. At various stages in the process of the foodstuff breakdown, the products formed may not only take on or give off a molecule of water or of phosphate, but also lose a molecule of CO_2 by a decarboxylation reaction. The end products of the reaction then are CO_2 and water. Note, however, that all of the molecular oxygen that participated in the reaction appears as water and none goes directly to form CO_2 . The oxygen that finally appears in the CO_2 was either in the foodstuff to start with or is oxygen that was added as water (or phosphate) during the reactions. We shall return to this point later.

It should be noted also that though all of the oxidative enzymes participate in the passage of electrons from foodstuff to oxygen, they all do not participate in the passage of hydrogen ions. The cytochrome members of the chain transfer only electrons and, moreover, they are able to transfer only one electron at a time. The flavoproteins and pyridine nucleotides, on the other hand, transfer both hydrogen ions and electrons and are capable of transferring a maximum of two electrons at a time. The hydrogen atoms that combine with oxygen to form water must thus be obtained by combining electrons passed through the oxidative enzyme chain with hydrogen ions withdrawn from the environment.

Now during each of these many reactions, energy has been liberated so that the next question we may ask is what proportion of the total energy can be accounted for in each one of these reactions. In order to answer this question, let us focus our attention more closely on the oxidative enzymes which are participating in these reactions. Each one of these compounds is capable of undergoing a reversible oxidation and reduction and, therefore, forms a system whose oxidation-reduction

potential should be measurable in relation to any other system. Unfortunately, the direct measurement of the oxidation-reduction potential of most of these systems is fraught with technical difficulties that need not, however, be detailed here. Let it suffice to say that it has been possible to obtain at least approximate values for all of them except cytochrome oxidase. In TABLE 1, the oxidation-reduction potentials of these systems along with those of the oxygen and hydrogen electrodes at pH 7.0 are listed. It will be noted that the potentials of these systems decrease progressively in the order listed, and it is this relationship which formed the basis for their arrangement in FIGURE 1. In this connection, it is highly important to mention the fact that these systems show a marked specificity of interaction where this can be tested. For example, the reduced pyridine nucleotides will not react directly with oxygen nor with cytochrome c, but only with flavoprotein. Though the evidence is not complete, we shall assume each compound listed here will react only with the compound listed immediately above or below it. We may now calculate the potential differences between neighboring pairs of these systems and obtain the free energy released on their interaction by means of the equation

$$\Delta F = -nFE$$

where n equals the number of electrons exchanged, F is the Faraday, and E is the potential difference expressed in volts. Taking the Faraday as equal to 23,068 calories, we obtain the free energy values given in the last column of TABLE 1.

Inspection of this table indicates that the number of calories released varies greatly, depending upon the pair of systems which are interacting. For example, the interaction of cytochrome a and cytochrome c, according to these calculations, yields only 460 calories while 9,200 calories are released when two electrons are transferred from the pyridine nucleotides to flavoprotein. Are we now to conclude that these values represent the maximum energy to be obtained upon the interaction of these systems in the animal body? The answer is in the negative, of course, because our calculations are based upon the E' values of these systems and thus represent only the amount of energy released upon the interaction of these systems when they are all 50 per cent oxidized (or reduced). It is doubtful if this is the relationship between them during the metabolic activity of the cell. It is possible, therefore, that more, or even less, energy is released in the living cell by the interaction of any pair of these systems than is calculated here. Unfortunately, we have no information which will permit us to say pre-

TABLE 1
 E'_0 AND ΔF VALUES FOR THE INTERACTION OF THE OXIDATIVE
 ENZYMES AT pH 7.0 AND 30° C.

	E'_0	ΔF per electron	
O ₂	+0.81 v	0.52 v	-12,000
Cytochrome oxidase	?		
Cytochrome a	+0.29 v	0.02 v	-460
Cytochrome c	+0.27 v		
Cytochrome b	-0.04 v	0.31 v	-7,150
Flavoprotein	-0.08 v		
Pyridine nucleotide	-0.28 v	0.04 v	-920
		0.20 v	-4,600 ($\times 2 = -9,200$)
H ₂	-0.42 v		-25,130 cal.

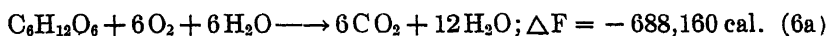
$$\Delta F = -nFE = -1 \times 23,068 \times \text{voltage difference}$$

The source of the E'_0 values of all systems except the pyridine nucleotide is given by Ball.¹ The value for this system is that calculated by Borsook.⁶ The E'_0 value for this system of -0.26 v reported by Ball and Ramsdell² is undoubtedly too high, since subsequent tests have indicated that the sample of pyridine nucleotide employed by them was not 100 per cent pure.

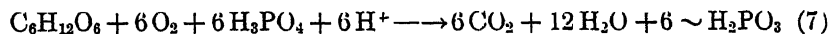
cisely what amount of energy is released by the interaction of any pair of these systems in the living cell. At best, the calculations given here are useful only to indicate the rough magnitude of possible energy release in the interaction of these various systems in the animal body. For example, if we accept 12,000 calories as the energy necessary to form a high energy phosphate bond, it would seem reasonable to suppose that the reactions most likely to furnish this amount of energy would be those between the pyridine nucleotides and flavoprotein (two electrons), cytochromes b and c, or of cytochrome oxidase with either O₂ or cytochrome a, depending on the potential of the cytochrome oxidase system. In any one cycle, of course, all three of these pairs could not each supply 12,000 calories, since, to obtain 12,000 calories from any one of these pairs, would mean a corresponding decrease in energy release elsewhere in the chain, on the basis of the calculations in TABLE 1. By summation of the energy release for all of these systems, we see that only some 25,000 calories are capable of being released for each electron transferred through the total chain so that, no matter how we choose to subdivide it, this is the total amount of energy available per cycle. In view of this fact and the number of participating systems in the chain, it seems not unreasonable to assume that the 12,000 calories required to form a high energy phosphate bond represent about the maximum individual parcel of energy to be drawn off

from oxidative reactions. However, it must not be forgotten that cytochrome oxidase, the three cytochromes, and the flavoprotein reacting in this chain are all apparently intimately associated in a submicroscopic particle within the living cell. It is possible, therefore, that energy release should not be discussed in terms of pairs of these systems, since it is conceivable that a close association of these enzymes permits energy exchanges to occur that might not otherwise be possible. We shall be on more substantial ground if we deal with the total amount of energy released by interaction of all of the oxidative enzymes in the chain during the passage of one electron from the half reduced pyridine nucleotide system to oxygen.

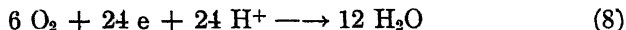
Let us then return to the oxidation of glucose and attempt to calculate what percentage of the total free energy of this reaction will be released by the interaction of the oxidative enzymes. We have seen that, in any biological oxidation, all the molecular oxygen that enters the reaction will appear as H_2O . Therefore, in the oxidation of glucose in the living cell, we will write the equation



so that the six oxygen molecules that enter the reaction will produce 12 H_2O . If this equation is to balance, however, we must write 6 H_2O on the left-hand side and we may, therefore, say that at least 6 water molecules enter into the intermediate reactions which take place when glucose is oxidized in the animal body. An alternative method would be to write the equation as involving the participation of phosphoric acid with the production of high energy phosphate bonds as in equation (7).



Parenthetically, it must be pointed out that this equation must not be interpreted as defining the limits for the number of high energy PO_4 bonds formed per mole of glucose burned. Regardless, however, of the manner in which we write this equation, the main fact that concerns us here is that the conversion of 6 molecules of oxygen into water requires the participation of 24 electrons and 24 hydrogen ions.



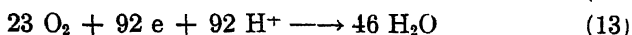
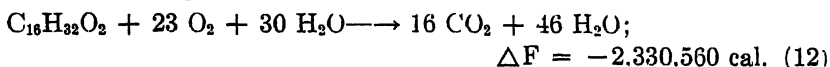
This means that 24 electrons must be transferred from the substrate through the oxidative enzyme chain to O_2 for each mole of glucose burned in the body. Now, if we assume that all the energy released is by electron transfer, we may calculate that the maximum free energy that could be released per electron transferred would be the total free

energy of 688,160 calories divided by 24, or 28,670 calories. To be sure, all energy release is probably not by electron transfer, but since we have presented in TABLE 1 calculations showing the amount of free energy released by the exchange of one electron between the various oxidative enzymes, we are now able to obtain some idea of the proportion of the total energy that may be so released. For example, the transfer of one electron from the pyridine nucleotides to O_2 was calculated to release 25,130 calories. A simple calculation shows this to be 87.6 per cent of the total maximum free energy theoretically available for release. It must be pointed out, however, that this value represents nearly the maximum percentage of the total free energy that could be released by the passage of electrons through the chain of oxidative systems. Actually, somewhat less than this amount may be so released, because we know that the potential of the pyridine nucleotides lies below some of the reversible substrate systems with which they react.

In all fairness, then, let us take the next enzyme system in the chain, flavoprotein, and repeat the calculations. The transfer of one electron from flavoprotein to O_2 can be calculated to release 20,530 calories and this represents 71.6 per cent of the total free energy available. It seems reasonable to assume that this represents the minimum possible percentage of the total free energy released through the oxidative enzymes since, at the E'_0 value of the flavoprotein system, the ratio of oxidized pyridine nucleotide to the reduced form would be about five million to one. Thus, we reach the conclusion that somewhere between 72 and 88 per cent of the total free energy released during the oxidation of glucose in the animal body can be accounted for by interaction of the oxidative enzymes. The remainder of the energy, amounting to 12-28 per cent of the total, can then be said to be released during the interaction of the substrates with the pyridine nucleotides, or in reactions in which the removal of electrons and hydrogen ions is not involved. A good example, of course, of the latter type of reaction is the anaerobic conversion of glucose into 2 lactate molecules. Here, there is merely a molecular rearrangement without the loss of any electrons, and it can be calculated that under body conditions some 50,000 calories per mole of glucose are liberated by the reaction. To be sure, diphosphopyridine nucleotide participates in this reaction, but it acts merely as a shuttle to redistribute electrons and hydrogen ions. Thus, the conversion of glucose into lactate yields only some 7 per cent of the total free energy to be obtained by complete glucose combustion. It is interesting to note that, even after such an anaerobic energy release, the prod-

uct of the reaction, lactate, still is oxidized by the same pathway involving a pyridine nucleotide. Now, if all products of anaerobic reactions must still lose their electrons by this pathway, then we may conclude that the maximum anaerobic free energy release for glucose under any circumstances cannot be greater than 12-28 per cent of the total free energy released on oxidation.

Similar calculations may also be made for the combustion of a fatty acid. Using palmitic acid as an example, the equations given here summarize the pertinent data.



$$-2,330,560 \text{ cal.} \div 92 \text{ e} = -25,332 \text{ cal. per electron transferred} \quad (14)$$

One electron from pyridine nucleotides to $\text{O}_2 = -25,130 \text{ cal.}$

$$\frac{25,130 \text{ cal.}}{25,332 \text{ cal.}} = 99.2 \text{ per cent of total energy} \quad (15)$$

One electron from flavoprotein to $\text{O}_2 = 20,530 \text{ cal.}$

$$\frac{20,530 \text{ cal.}}{25,332 \text{ cal.}} = 81.0 \text{ per cent of total energy} \quad (16)$$

If the oxidation of palmitic acid follows the pathway we have outlined in FIGURE 1, then we may say that some thirty molecules of water must enter the reaction and 92 electrons will be transferred through the oxidative chain for each mole of acid oxidized. A total of 2,330,560 calories will be released as free energy, or a maximum of 25,332 calories for each electron transferred. By means of calculations similar to those used for glucose, we reach the conclusion that somewhere between 81 and 99 per cent of the total free energy would be released by means of the interaction of the oxidative enzymes when 1 mole of palmitic acid undergoes oxidation in the animal body. This leaves only 1 to 19 per cent of the total free energy to be released by other mechanisms, which is considerably less than was found in the case of glucose. Since we have postulated that it is from this portion of the total free energy that anaerobic processes should spring, it is interesting to speculate that perhaps this narrower margin in the case of fatty acids accounts for their apparent inability to furnish energy by anaerobic reactions.

I should perhaps pause at this point to acknowledge the fact that we are not by any means certain that the oxidation of all foodstuffs follows the outlined pathway. This is especially true of the fats, where our knowledge of the actual mechanism involved in their oxidation is in-

deed paltry. We do know, however, that some 90 per cent of the overall respiration of the cell may be blocked by cyanide, and this suggests that the cytochrome-flavoprotein chain is indeed responsible for the bulk of all oxidative processes. The 10 per cent cyanide insensitive respiration would appear to be due largely to flavoprotein systems, such as d-amino acid oxidase and xanthine oxidase, which are capable of direct reaction with O_2 . Whether any of the energy released by the action of such flavoprotein systems is utilizable by the organism remains to be answered. However, the potential of such flavoprotein systems is probably not greatly different from that of the flavoprotein participating in the cytochrome chain, so that the bulk of the energy release still occurs in the transfer of electrons and hydrogen ions between a flavoprotein and oxygen. It may well be, of course, that oxidative processes still unknown to us, and whose pattern is markedly different from the dehydrogenation type so familiar to us today, do occur in the body. If so, I can only hope that the deductions made here will so stimulate you to prove me wrong that the discovery of such mechanisms will be greatly hastened.

The best conclusion that we can, therefore, reach today is that the energy set free during the oxidation of foodstuffs is probably released in definite-sized parcels, a step at a time, with the bulk of the energy being released by the interaction of the oxidative systems. Whether the cell can further subdivide these parcels of energy for its use, we do not know. One interpretation of the all-or-none law of muscle and nerve action might be that these cells can respond only by expending a full parcel of energy whenever their threshold of stimulation is reached. Even so, the minimum size of this energy parcel is unknown. We also have little or no information to tell us whether the energy released at each one of these steps is capable of being harnessed for work by the cell. In some steps, for all we know, the energy may appear as heat to be wasted or utilized to maintain body temperature. Most of the information that we possess concerning energy utilization revolves around the high energy phosphate bonds formed in glycolytic reactions, and this topic lies outside my province. We may, however, garner some clues as to whether the energy released by the interaction of the oxidative enzymes is utilized by an inquiry into the efficiency of the living cell in performing its various functions. For this purpose, let us examine the relative efficiencies of certain different physiological processes.

The process of secretion or excretion is one in which certain calculations concerning efficiencies of energy utilizations may be made. Bor-

Book and Winegarden⁶ have calculated that the formation of one liter of urine requires about 700 calories. These same authors⁶ have calculated the efficiency of the kidney by assuming that a liter of urine is formed in 24 hours and that the total oxygen consumption of the kidney in this period of time furnishes energy which is all expended in the process of excretion. They reach the conclusion that the kidney performs its work with an efficiency probably not greater than 1-2 per cent. I have made similar calculations for the formation of pancreatic juice based solely upon the difference in its ionic composition and that of plasma and have obtained a value of 133 calories required for each liter of juice formed. Using the data of Still, Bennett, and Scott¹⁰ for the difference in the O_2 consumption of the resting and secreting gland, it is possible, assuming an R.Q. of 1.0, to calculate that, in the formation of 1 liter of pancreatic juice, approximately 3800 calories are consumed. The efficiency of secretion of pancreatic juice is thus of the order of 3-4 per cent. A similar calculation may be made for the gastric juice, and shows that about 1500 calories are required for each liter of juice formed. Data on the oxygen consumption of the gland are apparently not available, but if we assume that Davenport's⁷ explanation of the formation of the juice is correct, then one mole of CO_2 is produced for each mole of H^+ concentrated. With an R.Q. of 1.0, we may calculate that the amount of oxygen consumed in the process would be equivalent to 17,000 calories, and hence the efficiency of the process is of the order of 9 per cent. We thus reach the conclusion that the over-all efficiency of processes of excretion or secretion lies within the range 2-10 per cent. It is thus possible for us to postulate that, in such processes, either (1) that all the various steps in the oxidative process contribute inefficiently to the supply of the energy requirements, or (2) that the energy of only certain reactions can be used efficiently and that the rest is wasted. Whether the interaction of the oxidative enzymes can furnish energy for such processes is thus, at the present time, debatable.

We may next consider a process in which energy is converted into mechanical work. Benedict and Cathcart⁴ have presented data to show that, in man, muscular work may be performed with an efficiency as high as 20 per cent. Now, we have seen that, on the average, only some 20 per cent of the total energy released by the oxidation of food-stuffs can be attributed to reactions not involving the oxidative enzymes. We must, therefore, conclude that either such processes are 100 per cent efficient, or that some of the energy released by the inter-

action of the oxidative enzymes is utilizable. A consideration of the over-all efficiency of oxidative phosphorylation processes makes the latter explanation seem most reasonable.

Belitzer and Tsibakowa³ and more recently Ochoa⁸ have reported that during the oxidation of carbohydrate by heart muscle, as many as six energy-rich phosphate bonds may be formed for each molecule of oxygen consumed. Accepting 12,000 calories as the energy needed to form one energy-rich phosphate bond, we may calculate that, in the combustion of one mole of glucose where 6 moles of oxygen are utilized, some 36 such bonds representing 432,000 calories will be formed. This corresponds to some 63 per cent of the total free energy released upon the oxidation of one mole of glucose. Hence, the conclusion seems inescapable that some of the energy released by the interaction of the oxidative enzymes is being utilized to form high energy phosphate bonds. Thus, the 20 per cent over-all efficiency of muscular work must be attributed to energy wastage during the muscle's utilization of phosphate bond energy as well as in its inefficient use of energy from oxidative processes.

In summary, then, it would appear from our present-day knowledge that the bulk of the energy liberated in the oxidation of foodstuffs is released by the interaction of the oxidative enzymes and that some of the energy so released may be utilized by the living organism. However, many puzzling questions still remain to be answered. There is lacking any knowledge of the factors which control the release of energy by such processes to meet the fluctuating needs of the cell. We are also in the dark concerning the mechanisms by which the energy released is geared to useful purposes within the cell. The vitamins have already been brought into the picture, perhaps the hormones are next. Surely, exciting discoveries lie ahead for those who are delving into these problems, and we may await future developments in this field with great expectancy. May the day soon come when our undivided efforts can again be directed toward the solution of such problems.

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ENERGY RELATIONSHIPS IN GLYCOLYSIS AND PHOSPHORYLATION

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Our knowledge of the energy relationships in glycolysis and phosphorylation developed in close connection with the problem of the energy-transferring chemical reactions of muscle activity. I may be allowed, therefore, to start with that topic. Leaving aside the concepts of the so-called classical period of physiology and starting with the myothermic work of Hill and Hartree about 1920, we may broadly distinguish three historic periods in these investigations.

In the first period, which fills a little more than a decade from 1920 to 1932, the relationship of the anaerobic muscle metabolism to the oxidative metabolism, as well as to the production of heat and work, was studied in the living isolated muscle. As a result, the three most important reversible systems connected with muscular activity were brought to light: (1) Splitting of carbohydrate to lactic acid and aerobic resynthesis; (2) splitting of creatinephosphate into creatine and phosphate and anaerobic and aerobic resynthesis; (3) splitting of adenylypyrophosphate and anaerobic and aerobic resynthesis.

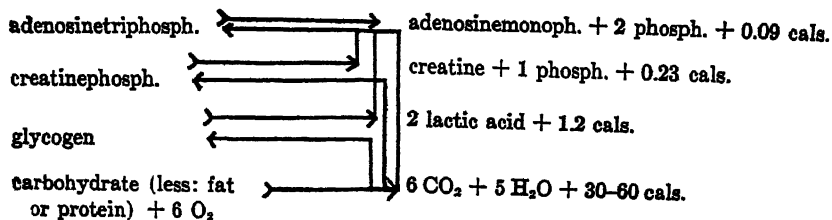
In the second period, by a close study of the enzymatic breakdown of carbohydrate in solution, the intermediates in this breakdown were isolated, transfer of phosphate and hydrogen could be ascribed to single reaction steps, and the equilibria in solution could be established, as well as the total energy of the different steps. This period again roughly fills a decade and closes with the latest discovered intermediate, 1,3 diphosphoglyceric acid, by Warburg, Christian and Negelein in 1939.

The final period, in which we now stand, thus far has shown three different trends: firstly, to develop a more general theoretical view of the energy transfer by means of energy-rich phosphate bonds, especially in the contributions of Lipmann¹ and Kalckar² to this subject; secondly, to generalize the relation between hydrogen transfer and phosphorylation to all oxidative steps, that is, to the respiration of carbohydrate as well as to the oxidoreduction. In addition to the work of the above investigators, one may mention several papers from Cori's laboratory³ and also some by Ochoa.⁴ The third tendency has been to apply our knowledge to a final understanding of the contraction mechanism of muscle by means of the hypothesis that the phosphate-bond energy is finally transferred to the contractile protein itself. I

here refer to the work of Engelhardt, followed by that of J. Needham and others. Although these attempts, at the moment, are highly speculative, eventually they may lead to the clue to this last energy transfer. I shall restrict my report mainly to the first two periods, in which I was able to participate more actively. I expect that Dr. Kalckar, as a representative of a younger generation, will devote more of his time to the more recent developments.

Before going into details, I may state that the heat of the different reactions can be measured by direct calorimetry with considerable accuracy,—much more accurately than by calculation from measured combustion heats, which involve small differences between large values. The latter method is subject to great errors, especially if the heats of solution and dilution are not known or if the effect of phosphorylation is disregarded. But in some cases, we must rely on the latter procedure, where, for any reason, direct calorimetry is inapplicable. All energy-rich phosphate bonds were found by direct calorimetry. On the other hand, the change of free energy in the reaction can be measured directly only in those instances where the equilibrium is not shifted too far to one side (with the proportion of the reactants not higher than 99:1), so that the equilibrium concentrations can still be accurately measured. This corresponds, for monomolecular reactions, to K values between 10^2 and 10^{-2} ; for bimolecular splitting or synthesis, to K values between 10^4 and 10^{-4} . For our ranges of temperature and concentration, ΔF is then always smaller than approximately 5000 calories. Since phosphorylation potentials cannot be determined by means of electromotive force measurements like oxidation potentials, free energy changes of those types of phosphate transfer which yield great energy ($-\Delta F$ over 5000 calories), and which are more interesting, must be calculated more or less with the help of thermal data.

I shall now come to the first historic period of which I have spoken. How are we to picture, with our present-day knowledge, the over-all energy exchange of a working muscle under aerobic and anaerobic conditions? Here the general scheme which summarizes the results of this period still holds.⁵



The calories here refer to the available energy stores for the different reactions in one gram of unfatigued frog muscle. Every reaction below that shown in the uppermost line of the diagram is farther away from the immediate supply of energy for work, and has, at the same time, a larger store of energy. Moreover, it is coupled with the reversal of the adjacent reaction, that is, with the recombination of the split products. But it is also coupled with all others by shunts. This is very important. For instance, the oxidation of carbohydrate can bring about the synthesis of creatine-phosphate not only by way of intermediate lactic-acid formation, but directly as well. Under strict aerobic conditions, this applies not only to the alactacid muscle poisoned with iodoacetic acid^{5a} but to the normal muscle as well. Such a muscle, apparently, does not form lactic acid at all, and the oxidation serves for immediate resynthesis of creatinephosphate. Lactic acid formation is already an emergency mechanism, which, however, is set in motion under normal conditions in a muscle working *in situ*; especially, until the blood supply is adapted to the sudden increase of the demand of oxygen by the start of work. Intermediate lactic acid formation, therefore, is in response to the lack of oxygen, while breakdown of creatinephosphate in the working muscle occurs alike in presence and absence of oxygen.

This view is somewhat different from the older assumption that, even under normal oxygen tension, lactic acid appears as an intermediate in the active non-poisoned muscle; and it removes a curious difficulty or paradox encountered in the relationship of phosphocreatine breakdown and lactic acid formation during anaerobic fatigue of an isolated muscle.⁶ In the first contractions of such a series of twitches

or tetani, the quotient of $\frac{\text{creatinephosphate split (mole)}}{\text{lactic acid formed (mole)}}$ is high,—

about 3 to 4. For high degrees of fatigue it is very low, probably less than 0.2. But "creatinephosphate split" in the numerator refers to that residual amount of split creatinephosphate which is found by analysis after the contraction, and which is not immediately resynthesized during lactic acid formation. From this, it must be concluded that, at first, extremely small amounts of creatinephosphate are resynthesized by coupling with lactic acid formation and, in high degrees of fatigue, nearly all newly split creatinephosphate is synthesized in that way. This paradoxical result, that anaerobic resynthesis of creatinephosphate becomes more and more evident with increasing anaerobic fatigue, and is nearly absent in the beginning, is easy to understand when one considers that the mechanism of coupling with lactic acid is only an emer-

gency device. Usually creatinephosphate is synthesized by coupling with *oxidation*. But when a larger part is broken down and oxygen is not available, lactic acid formation sets in, which counteracts the depletion of the energy store of creatinephosphate. Theoretically, it would seem possible that muscle contraction could proceed in oxygen solely with the breakdown of adenosinetriphosphate and the subsequent resynthesis, without intermediate breakdown of creatinephosphate. The available store of .09 calories would be enough for about 30-50 single maximal contractions. But by registration with glass electrodes from the muscle surface, Dubuisson^{7a} has obtained a curve of pH change, which shows an alkalization even in the very first contractions, and this alkalization is interpreted as a breakdown of phosphocreatine.

From these newer concepts of muscle metabolism, we can expect an approximately constant caloric quotient of lactic acid in muscle, calories produced anaerobically, only with similar degrees of anaerobic gms. lactic acid formed

fatigue. Although a quite short activity of muscle is unsuited for chemical analysis, relatively short periods of stimulation formerly gave caloric quotients of about 400 calories, while, in the highest degrees of fatigue, the quotients found were 280 to 250 calories. Since 205 calories are developed enzymatically by splitting 0.9 grams of dissolved glycogen into lactic acid [180 calories (difference of combustion heat) + 25 calories (heat of neutralisation with bicarbonate and phosphate)], the minimum of 250 calories corresponds to nearly exclusive lactic acid formation with an approximate balance of split and resynthesized creatinephosphate, and with some additional heat derived from the great neutralization heat of protein.* The higher caloric quotients correspond to additional breakdown of creatinephosphate. When the molecular heat for the enzymatic hydrolysis of creatinephosphate (11,000 calories per mole) is taken as the basis, and the muscle is analyzed for both lactic acid and creatinephosphate, it seems that the total heat is still somewhat greater than explained by this breakdown, even if the pH change and the large neutralization heat of protein is taken into account.^{8a}

Finally, we may ask how the myothermic measurements of Hartree and Hill can be explained for single twitches of a muscle in oxygen when only creatinephosphate is split, as in the case of muscle poisoned by iodoacetic acid. According to Hartree and Hill, about 50 per cent

* The heat of neutralization of protein amounts to 12,500 calories per equivalent, or 140 calories per gm. of lactic acid, but probably very little is neutralized in this way.

of the total heat of contraction is given up anaerobically in the initial phase during contraction, while 50 per cent is delayed heat in oxygen. For each mole of oxygen, burning carbohydrate, 113,000 calories are developed; and for each mole of creatinephosphate hydrolyzed, 11,000 calories are liberated. Therefore, 5 moles of creatinephosphate must break down and be resynthesized by one mole of oxygen. This would give 55,000 calories for the initial heat, and $113,000 - 55,000 = 58,000$ calories for the oxidative heat. Actually, Nachmansohn and I, in 1930,⁹ found between 3.6 and 5.4 moles of creatinephosphate (averaging 4.7 moles) resynthesized for each mole of oxygen consumed, during the first part of the recovery period after short stimulation, when about 5 times as much creatinephosphate synthesized is about 5 times the lactic acid which disappears. In the minced muscle, Belitzer and Tzibakowa¹⁰ found, that when three- or four-carbon acids were oxidized, 4 moles of creatinephosphate were resynthesized for each mole of oxygen consumed. Theoretically, on the basis of known mechanisms of phosphate transfer, the relation of 4 moles of phosphate to one mole of oxygen can be understood, while, at the present time, higher proportions cannot be interpreted by means of known coupled reactions. But, nevertheless, they seem to occur, since recently Ochoa⁴ has reported the transfer of phosphate to sugar in heart extracts in the proportion of 6 moles of phosphate to each mole of oxygen. From a thermodynamic viewpoint, such high yields of esterified phosphate per mole of oxygen are possible, even if the phosphate should be stored in an energy-rich linkage as in creatinephosphate, and would not form the usual phosphoric acid ester. Oxidation during the recovery period in the isolated amphibian muscle has no higher efficiency than 50 per cent, equivalent to the creation of 4 or 5 energy-rich phosphate bonds per mole of oxygen. But, since we know that anaerobic glycolysis has an efficiency of 100 per cent in creating such phosphate bonds, special means may exist in respiration, to bring this about, such as, for instance, the large gap of oxidation reduction potential between the cytochromes and the pyridine nucleotide.

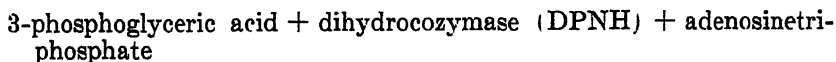
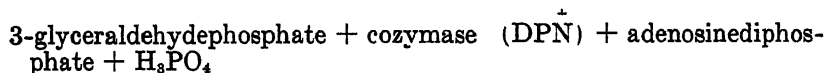
The second part of my report is concerned with the results of the second historic period of our problem. In this period, the whole pathway of breakdown leading from glycogen to lactic acid was cleared up by studying the intermediate reactions in muscle extract, yeast extract, in other cell extracts and more or less isolated enzymatic systems. Two general reaction types became known. The first includes those reactions easily reversible in the absence of other additional chemical systems,

which therefore display a small change of free energy. These can be called, in a special sense, "equilibria reactions." The second type includes those, which can be reversed only by coupling with a second system, where every single reaction shows a relatively great change of free energy. But, by the combination of inducing and induced reaction, a reversible system can be built up, which is nearly "ergo-neutral." In general, the first type of reaction goes on in dialyzed extracts, where the coenzyme systems are removed, while the latter type needs coenzymes, which form a part of the coupled systems. In the stationary state of lactic acid formation from glycogen, about twelve consecutive steps are passed through, of which eight belong to the first-named group, while the rest are so combined that the change of free energy therein is equal to the change of free energy in the total chain of reactions from glycogen to lactic acid. By coupling with the adenylic system as the phosphorylating coenzyme, this free energy is transferred to two energy-rich phosphate bonds per mole of lactic acid produced.

I shall not describe here the single reactions, because I have done this previously¹¹ and they are well-known. However, some of the equilibrium reactions, undoubtedly, are very interesting from chemical and thermodynamic viewpoints. I mention, for instance, the zymohexase reaction between hexosediphosphate and triosephosphate, which has a strong negative heat in the direction of splitting and obeys closely the Van't Hoff law of isochores. I shall restrict myself to the reactions of the second type, with appreciable yield of free energy, and shall only discuss those points where either some progress has been made in recent times or the situation is still somewhat obscure. The concept of the energy-rich phosphate bond, developed mainly by Lipmann,¹ has undoubtedly helped to clarify and systematize our knowledge in this field. At present, it will be generally agreed by all experts in this matter, that the significance of the phosphorylation of the carbohydrate intermediates is based on its thermodynamic implications. In this way, the energy of the oxidative step can be easily collected in energy-rich phosphate bonds, which, at first, form a part of the intermediates themselves. By transphosphorylations, these bonds with their high energy are transferred to the adenylic system and are stored as the labile phosphate groups in the adenosinepolyphosphoric acids or are transferred still further to creatine and are stored as creatinephosphate. They can also be transferred to other systems with or without loss of their high bond-energy or possibly they can be released, when attached to protein in doing mechanical work.

By direct measurement, we know only the heat change connected with the release of such an energy-rich bond. The molar heat for the splitting of one labile group of adenosinetriphosphate is about 12,000 calories; for creatinephosphate, it is 11,000 calories; for phosphopyruvic acid, it is 8000 calories; and for argininephosphate, also 8000 calories. Lipmann has found an indirect method for calculating the free energy change of the dephosphorylation of phosphopyruvic acid and has obtained a ΔF° of $-11,000$ calories. Assuming that the free energy is nearly the same in all energy-rich bonds, because of their mutual equilibria, this would be the true value for the other compounds too. Since a part of the calculations of Dr. Lipmann is based on somewhat doubtful numerical values, it may be of interest to submit a new computation of this ΔF° value of the phosphate bond of adenosinetriphosphate itself, from equilibria measurements made in my former Institute in Heidelberg in 1938. The equilibria measurements were published, but, so far, neither I, nor anybody else, has attempted to use them for this calculation. All figures (except one for the combustion heat of glyceric acid) are completely independent of Dr. Lipmann's numerical data. Indeed, the thermal values used by me contain uncertainties similar to those in Dr. Lipmann's computation. Nevertheless, since this calculation is based on quite different reactions and leads to about the same result, it may add some more weight to the soundness of our assumptions.

The coupling reaction, which was described in 1938,¹² shows a stoichiometric balance, in which one mole of glyceraldehydephosphate is oxidized by one mole of cozymase to 3-phosphoglyceric acid, while one mole of phosphate combines with one mole of adenosinediphosphate to form adenosine triphosphate.



The equilibrium of this reaction was studied in a large series of experiments with different concentrations of the reactants. Although it was demonstrated by Warburg and Christian¹³ and also by Negelein and Brömel¹⁴ that this reaction is really composed of several steps leading over 1, 3-diphosphoglyceric acid, as a connecting link between oxidation and phosphorylation, this does not matter for the calculation of the

change of free energy, which is determined solely by the concentration of the initial and final products in the over-all equilibrium.

I have reproduced here the three most exact experiments, with their equilibrium concentrations. They were made under identical conditions of volume, temperature, etc., and with the same pure preparations of the different reactants. Experiment I, where all participants were used in about equivalent amounts, was made from both sides of the equation and reached nearly the same equilibrium point in both ways. It may, therefore, be taken as the most reliable. Experiment II was made only from the left side of the equation with the same initial concentrations, but with 10 millimole excess of 3-phosphoglyceric acid (PGA). Experiment III was made from the right side with 10 millimole excess of inorganic phosphate. Dihydrocozymase was determined spectrographically. In experiment I, the other components were all determined separately; in experiments II and III, they were partly derived from the value of dihydrocozymase. Glyceraldehydephosphate (GAP) is calculated from the zymohexase equilibrium.¹³ The agreement of the K value is as good as can be expected.

EQUILIBRIUM OF THE "COUPLING REACTION"*

pH, 7.8; temperature, 20° C.

(I) $K_e =$

$$1.27 \cdot 10^{-5} \text{ DPNH} \times 1.05 \cdot 10^{-3} \text{ PGA} \times 1.16 \cdot 10^{-3} \text{ ATP}$$

$$\frac{2.13 \cdot 10^{-3} \text{ DPN}^+ \times 0.04 \cdot 10^{-3} \text{ GAP} \times 2.6 \cdot 10^{-1} \text{ H}_3\text{PO}_4 \times 2.45 \cdot 10^{-3} \text{ ADP}}{= 2.8 \cdot 10^3}$$

(II) $K_e =$

$$0.62 \cdot 10^{-3} \text{ DPNH} \times 10.7 \cdot 10^{-3} \text{ PGA} \times 0.60 \cdot 10^{-3} \text{ ATP}$$

$$\frac{2.79 \cdot 10^{-3} \text{ DPN}^+ \times 0.045 \cdot 10^{-3} \text{ GAP} \times 3.2 \cdot 10^{-3} \text{ H}_3\text{PO}_4 \times 3.0 \cdot 10^{-3} \text{ ADP}}{= 3.2 \cdot 10^{-3}}$$

(III) $K_e =$

$$1.74 \cdot 10^{-3} \text{ DPNH} \times 1.74 \cdot 10^{-5} \text{ PGA} \times 1.74 \cdot 10^{-3} \text{ ATP}$$

$$\frac{1.92 \cdot 10^{-3} \text{ DPN}^+ \times 0.035 \cdot 10^{-5} \text{ GAP} \times 11.9 \cdot 10^{-3} \text{ H}_3\text{PO}_4 \times 1.9 \cdot 10^{-3} \text{ ADP}}{= 3.5 \cdot 10^{-3}}$$

For $K = 3 \cdot 10^3$, $\Delta F'^{\circ} = -RT \ln K = -1342 \times \log. K = -4700$ calories. In calculating from this equilibrium the ΔF° for $\text{ATP} \rightarrow$

* The data are taken from, (12) table V, pages 125 and 126. For experiment I, the intermediate values for the equilibrium concentrations attained from both sides are used.

ADP + H₃PO₄, I had the kind help of Dr. H. Kalckar, who especially assisted me to transform the thermal data of the reaction (phospho) glyceraldehyde + H₂O → (phospho) glyceric acid + 2H⁺ into values of free energy.

The total equilibrium of the coupling reaction may be divided into the three partial equilibria K₁, K₂, K₃, and their corresponding ΔF'^o values ΔF'^o₁, ΔF'^o₂, ΔF'^o₃, respectively.

$$K_1 = \frac{\text{DPNH}}{\text{DPN}^+ \times 2\text{H}^+} \quad K_2 = \frac{3\text{-Phosphoglycerate} \times 2\text{H}^+}{3\text{-Glyceraldehydophosphate} (+ \text{H}_2\text{O})}$$

$$K_3 = \frac{\text{ATP}}{\text{ADP} \times \text{H}_3\text{PO}_4} \quad \text{and} \quad \Delta F'^{\circ}_c = \Delta F'^{\circ}_1 + \Delta F'^{\circ}_2 + \Delta F'^{\circ}_3.$$

thus: $\Delta F'^{\circ}_3 = \Delta F'^{\circ}_c - \Delta F'^{\circ}_1 - \Delta F'^{\circ}_2$

ΔF'^o₁ can be taken from the value of Borsook¹⁰ for E'^o (—0.28 volt at pH 7) of this equilibrium. When corrected for pH 7.8, this gives, in the endergonic direction, +13,500 calories. ΔF'^o₂ can be calculated only tentatively with the help of not too accurate thermal data according to the equation: ΔF₂ = ΔH₂ — TΔS₂. In general, ΔH refers to the difference of the heat content of the pure substances. The subtrahend should include, besides the difference of the entropies (S) of the pure substances, the difference of the entropy changes in dissolving the substances at standard conditions (molar concentration, pH = 0). The terms for the ionization of the acid formed by oxidation and for shifting the pH from 0 to the pH of the equilibrium (7.8) finally must be added. In our cases, however, ΔH₂ corresponds to the difference of the heat content of the dissolved diluted substances, since only the heats of reaction of the dissolved phosphorylated compounds are known and not their heats of combustion. It would be entirely misleading to use instead of this the combustion heats of the unphosphorylated compounds without a thorough and critical evaluation. The combustion heat of the dimeric racemic glyceraldehyde¹⁷ cannot be reconciled with other heat measurements on trioses, as was pointed out earlier.¹⁹ The combustion heat of dioxycetone¹⁸ with the addition of the heat of solution is in better agreement with the value calculated from dissolved fructose and the endothermic reaction of the zymohexase: fructose-1, 6 diphosphate ⇌ 2 dioxycetonephosphate. Since the isomerisation of the triosephosphates is thermoneutral (ΔH > 1000 calories), the value of H for dissolved glyceraldehyde may then be compared with the calculated value of dissolved glyceric acid.¹ Other possible calculations are based on the measured heat of the dismutations: triosephosphate + acetaldehyde = phosphoglycerate + ethyl alcohol; and triosephos-

phate + pyruvate = phosphoglycerate + lactate.^{12, 20} In these cases the difference of the heat content of dissolved acetaldehyde and ethyl alcohol, and likewise of pyruvic and lactic acid must be subtracted. These values can be taken from known combustion heats and heats of solution and dilution. Moreover, one must take into account the concomitant phosphorylation of hexose in the first mentioned enzymatic dismutation, and of creatine in the second one, as well as the heat of neutralization. In the mean, ΔH for the reaction glyceraldehydophosphate + $H_2O \rightarrow$ phosphoglycerate + $2H^+$ turns out to be + 4000 calories (± 1500 calories).^{*} $T\Delta S$ for glyceraldehyde (phosph)/glyceric acid (phosph) in their pure state probably is small, judged from analogies in the table of Parks and Huffman²¹ (table 40, p. 210). The possible differences of the change of entropy by solution must be disregarded owing to lack of suitable data.[†] Thus the $T\Delta S$ value, which has to be taken into account, is that of the molecule of water which disappears: $T\Delta S_{(H_2O)} = \Delta H_{(H_2O)} - \Delta F_{(H_2O)} = -68,300 + 56,600$ calories = -11,700 calories. The ionization of glyceric acid to glycerate⁻ at pH 0 is endergonic. The pK of the glyceric acid group in phosphoglyceric acid = 3.42.²² Since $-RT \ln K = -1342 \cdot (-3.42) = +4600$ calories at 20° C, ΔF°_2 for glyceraldehydophosphate + $H_2O \xrightarrow[-3H^+]{-2e} \text{phosphoglycerate}^- = +4000 - 11,700 + 4600$ calories = -3100 calories.

The shift of the system of pH 0 to pH 7.8 follows at 20° C, a 58 millivolt slope, as far as pH 3.42 and an 87 millivolt slope from pH 3.42 to 7.8. This corresponds to -27,000 calories. Thus, $\Delta F'^{\circ}_2$ (pH 7.8) = -3100 - 27,000 calories = -30,100 calories. No correction is applied for the increase in strength of the second phosphate group of phosphoglycerate compared with glyceraldehydophosphate (pK 5.98 instead of pK 6.75),²² which possibly would add -1000 calories.

Inserting the ΔF values in the complete equation we get: $\Delta F'^{\circ}_3 = -4700 - 13,500 + 30,100$ calories = +12,000 calories. In the exergonic direction ($ATP \rightarrow ADP + H_3PO_4$), $\Delta F'^{\circ}_3 = -12,000$ calories.

^{*} For this calculation, the heat of formation of the glyceraldehyde group (dissolved) is taken as -140,700 calories, of water as -68,300 calories and of the glyceric acid group (diss.) as -205,900 calories. The values of ΔH s obtained by the four different methods of calculation are: From diacetyone (diss.), glyceric acid (diss.), and thermoneutrality of the isomerisation,^{19a} +3500 calories.

From fructose (diss.) \rightarrow hexosediphosphate \rightarrow 2 triosephosphate, glyceric acid (diss.),^{19a} + 5800 calories.

From the dismutation with acetaldehyde/alcohol,¹² + 5000 calories.

From the dismutation with pyruvate/lactate,²⁰ between +2500 and 4500 calories.

[†] From K_{isomerase} = 22 for glyceraldehydophosphate \rightarrow diacetyone phosphate it follows that ΔF of this isomerisation equals -1800 calories (in dilute solution). But this equilibrium does not enter the computation in the text.

This value is in good agreement with the value for $\Delta F'^{\circ}$ obtained by Dr. Lipmann for the dephosphorylation of phosphopyruvic acid, namely, $-11,250$ calories.

It is worth while to compare this total equilibrium of the coupling reaction with the intermediate equilibrium found by Warburg and Christian in the presence of cozymase and pure oxidizing enzyme:

$$K_{\text{oxidation}} = \frac{\text{DPNH} \times 1, 3\text{-diphosphoglyceric acid}}{\text{DPN}^+ \times 3\text{-glyceraldehydephosphate} \times \text{H}_3\text{PO}_4}$$

This equilibrium constant is strongly dependent on the pH, and at 7.8 and 20°C probably is about 3, only 1/1000 of that of the total equilibrium. Therefore, $\Delta F'^{\circ}$ for glyceraldehydephosphate + $\text{H}_3\text{PO}_4 \rightarrow 1, 3$ diphosphoglyceric acid is about $-14,000$ calories, since $\Delta F'^{\circ}$ for the oxidoreduction with DPN is only some 100 calories. We can deduce from this result that the consecutive reaction: 1, 3-diphosphoglyceric + ADP \rightarrow 3-phosphoglyceric + ATP, must have a $\Delta F'^{\circ}$ of about -4000 calories in order to obtain the $\Delta F'^{\circ}$ of the coupling reaction. Such a value, corresponding to a log K of 3 for the transphosphorylation would mean, that, with equal concentrations of the reactants, 3-4 per cent of diphosphoglyceric acid and ADP are in equilibrium with 96-97 per cent of 3-phosphoglyceric acid and ATP. Although it was announced at one time from Warburg's laboratory that the enzyme in question was isolated, nothing is known so far to verify this computation. I had, however, concluded from experiments on dephosphorylation, where this reaction was involved, that the equilibrium would be far to the right.²⁹

Let us now consider briefly the implications of the high phosphate-bond energy liberated by splitting off of phosphate, or absorbed by uptake of phosphate. In the oxidoreduction step, nearly as much of the free energy of the oxidation of glyceraldehyde to the glyceric acid level is taken up by phosphorylation of ADP to ATP, as is taken up for the reduction of DPN^+ to DPNH. The former is a net gain in free energy, while the dihydropyridine is reoxidized in the reduction of one mole of pyruvic to lactic acid. The free energy change of this reduction (pyruvate to lactate) at pH 7 is about $+8300$ calories. With a small loss in free energy, the reoxidation of dihydrocozymase reconverts the product of oxidation to lactic acid, that is, to the oxidative level of glucose.

Nature is still more skillful in accumulating the potential energy of glycolysis in phosphate-bond energy. The oxidation to glyceric acid makes possible the formation of the second energy-rich phosphate bond.

which is created by the dehydration of the intermediate 2-phosphoglyceric acid, to phosphopyruvic acid. This was already discussed some years ago by Lipmann.¹ The total heat change from glycogen to lactic acid amounts to 16,500 calories per mole of lactic acid without neutralization, or to 18,000 calories, with neutralization by ordinary buffer substances (like bicarbonate or phosphate). The creation of two energy-rich phosphate bonds would mean a gain in standard free energy of about 24,000 calories. This amount is about 30 per cent more than the total heat change—a very remarkable, but not miraculous result. Dean Burk had calculated, many years ago, that lactic acid formation in muscle under the conditions prevailing in the living organ could yield 50 to 80 per cent more free energy than total heat.²³

In the living muscle, two moles of creatinephosphate can be synthesized for one mole of lactic acid formed, which corresponds to two energy-rich phosphate bonds per mole. We identify the steps involved as the transfer of the carboxyl phosphate of 1,3-diphosphoglyceric acid and of the phosphate group of phosphopyruvic acid. Indeed, starting with hexosediphosphate, one easily obtains in enzyme extracts the synthesis of two moles of creatinephosphate per mole of lactic acid, where the over-all reaction is slightly endothermic and the energy available in the glycolytic splitting (18,000 calories per mole of lactic acid) is accumulated in the phosphate bonds of the two phosphocreatine molecules.²⁴ Nevertheless, the difficulty remains to be explained as to how, in the living muscle, hexosediphosphate has gotten its two phosphates. One phosphate group surely arises by means of the Cori reaction in the sequence: Glycogen + phosphate \rightarrow glucose-1-phosphate \rightarrow glucose-6-phosphate \rightarrow fructose-6-phosphate. But in the muscle extract, fructose-6-phosphate is phosphorylated to fructose-1,6-diphosphate only by means of the adenylic system, by consuming or wasting an energy-rich phosphate bond. Therefore, for one mole of hexose split, out of four energy-rich phosphate bonds, only three are completely comprehensible. If a reaction of the type, 2 fructose-6-phosphate \rightarrow 1 hexosediphosphate + 1 hexose, should occur, the difficulty in explaining the creation of the fourth phosphate bond would be overcome. But such a reaction is, so far, not known.

Without doubt, the formation of the carboxyl phosphate in 1,3-diphosphoglyceric acid is responsible for the autocatalytic formation of hexosediphosphate during the so-called phosphate period of cell-free alcoholic fermentation. Every molecule of hexosediphosphate which ferments not only regenerates a new one by means of its original content of two phosphate groups, but it also regenerates two by means of

this extra phosphate taken up during oxidation. The result corresponds to the snow-ball collection practice, formerly used in promoting charitable enterprises, where every contributor not only has to pay for himself, but, at the same time, to get a second man to pay the same amount and to agree to do likewise. How this "autocatalysis" is brought about in an enzyme extract is completely obvious now, but it is still a matter of controversy as to what, in the living yeast, controls the synchronization of phosphorylation and dephosphorylation, at least after a short initial period, in which, after addition of sugar, most of the inorganic phosphate in the cell is esterified. This controlling factor, apparently, is damaged or destroyed by killing the yeast.

In discussing this problem, I shall at first briefly mention the concepts of Nilsson, in Stockholm,²⁵ who still in 1942,²⁶ in a lecture which he gave during the present war in my former Institute in Heidelberg, deemed it especially suitable to attack my scheme of fermentation and to cling to his own old idea:—that sugar is not at all fermented by way of hexosediphosphate, but exclusively by way of hexosemonophosphate. The latter then should break down into one phosphorylated and one unphosphorylated triose molecule. In the living yeast, both parts would ferment; in extracts, only the unphosphorylated halves, and the phosphorylated portions would return to hexosediphosphate through the action of aldolase. A lipid structure in the living cell would be responsible for the fermentation of the phosphorylated triose and this structure would be damaged or destroyed by killing the yeast. The author inferred these assumptions from fermentation curves which he obtained from several types of dried yeast:—either the fermentation of sugar goes to completion with smoothly decreasing speed, or a break occurs after half of the sugar is fermented. This latter situation is the expression for the known equation of Harden and Young. In this case, provided there is less sugar present than its equivalent of inorganic phosphate, after fermentation of half of the added sugar, the other half is completely esterified to hexosediphosphate.

Some time ago, Warburg and Christian²⁷ backed this scheme of Nilsson's, but later, albeit tacitly, adopted my scheme. I think that it is not necessary to consider Nilsson's concept seriously in view of the bulk of the evidence that hexosediphosphate is the indispensable thoroughfare in the course of sugar breakdown. For those who are still intrigued by the slow fermentation of hexosediphosphate in yeast extracts in comparison with that of sugar, I mention only two sets of experiments which give a clue to this behavior. If one adds creatine to the maceration juice of yeast together with the phosphorylating en-

zyme from muscle, hexosediphosphate ferments to alcohol and carbon dioxide, at the same speed at which sugar ferments in the same extract. Creatine, which is foreign to yeast, serves now as the phosphate acceptor.^{24, 28} In a second type of experiment, arsenate may be added in about millimolar concentration. Under otherwise favorable conditions, hexosediphosphate here again ferments as rapidly as does sugar. In this case we assume, as do Warburg and Christian, that the coupling with phosphate uptake is interrupted by the formation of 1-arsenyl-3-phosphoglyceric acid, which splits off its arsenyl group without enzymes. I have shown recently how this explains, not only the rapid formation of phosphoglyceric acid, but also its rapid dephosphorylation.²⁹

There can be no doubt that sugar ferments completely by way of hexosediphosphate in the living yeast, as well as in the non-living yeast. Long ago, I had proposed tentatively as an explanation for the different kinetics in both cases, that the adenylypyrophosphatase is the most sensitive enzyme of the fifteen to twenty different partial enzymes of fermentation which form the zymase complex. By extracting or drying the yeast, it would be damaged more extensively than the others.³⁰ If this is true, one can assume that the adenylypyrophosphatase in the living yeast is sufficiently active for the regeneration of adenylic acid from adenosinetriphosphate, at the same speed at which hexosediphosphate arises anew from the oxidative coupling reaction with phosphate transfer to glucose. In this way, formation and dephosphorylation of hexosediphosphate would be synchronized. I admit that, so far, this idea is not definitely proved.* We shall, therefore, consider two other possibilities. Through the regeneration of hexosediphosphate, the energy-rich phosphate bond of adenosinetriphosphate is wasted. In the metabolism of muscle, this situation is avoided, because creatine takes up the energy-rich bond and stores it in the form of creatinephosphate. A continuous supply of energy-rich phosphate bonds is probably needed for many other synthetic purposes in the cell metabolism, as, for instance, for the formation of thiamindiphosphate from thiamin. The yeast cell and all other cells may contain very many such phosphate acceptors which, in a stationary state, would finally release the phosphate again by means of phosphatases, while, during growth, part of it would be preserved. I mention here briefly the related case of the autotrophic sulfur bacteria,

* I will mention, however, recent experiments which I performed to prove this point. We destroyed the yeast by freezing in liquid air or by ultrasonic vibration. The fermentation of hexosediphosphate by the extract of the cell fragments is increased many times by the addition of purified adenylypyrophosphatase from potatoes (O. Meyerhof, Jour. Biol. Chem. In press).

where, according to the highly interesting discovery of Umbreit, Vogler, *loc. cit.*,^{31, 31a} the energy of the sulfur oxidation is stored in adenosinetriphosphate and can be used anaerobically in the dark for the assimilation of carbon dioxide. Generally, synthesis may be mediated in this manner. This extra supply of energy-rich phosphate bonds in the stationary state of fermentation may, therefore, be of the greatest importance in nature. Probably such a mechanism is combined with the first-mentioned, and the adenylpyrophosphatase releases only the excess of that high energy phosphate not otherwise needed. Finally, the third possibility remains, that the living yeast makes use of a special enzyme for dephosphorylation of the carboxyl phosphate. This enzyme then would be destroyed or damaged by the extraction procedures. Such an enzyme is not known so far, but, if it exists, it would waste the energy-rich bond completely. Probably it would function similarly to the adenylpyrophosphatase in the last mentioned case. That this latter enzyme is preferentially and primarily injured by killing the yeast, is, after all, the most probable of these explanations.

When we look over the whole picture of the biological phosphorylation in carbohydrate breakdown, we arrive at some generalizations. I may cite some of these: Energy-rich phosphate bonds are only created, directly or indirectly, by the oxidative reaction steps in phosphorylated intermediates. On the other hand, all true ester phosphates, where the phosphate esterifies alcoholic groups, arise exclusively from transphosphorylation with the adenylic system or by intramolecular phosphate shift. Inorganic phosphate is never taken directly into alcoholic groups, but only into carbonyl or carboxyl groups. Probably the following generalization is also true: the function of the adenylic system as a dissociable coenzyme consists only in transphosphorylations, not in uptake or direct release of inorganic phosphate. This statement seems to contradict the important recent discoveries of Cori,^{32, 32a} that adenylic acid is a prosthetic group in the phosphorylase. The mechanism of this reaction, in spite of the abundance of discoveries made by Cori and his group, is, so far, not completely understood. Since adenosinedi- and triphosphate cannot replace the adenylic acid in the phosphorylase reaction, its function here must be different; moreover, it seems from the latest statements of Dr. Cori³³ that it may form a compound with another group, perhaps a dinucleotide, so that, in principle, the last generalization may also hold.

I have purposely dealt here only with what I called the first and second historic period, leaving to Dr. Kalckar to discuss the present-

day problems. These, indeed, are of more acute interest. On the other hand, much experimental material of the past years is still available for profitable interpretation and evaluation. A conference such as ours can promote such critical examination and coordination of thermal, electrical and chemical measurements, as may be necessary for drawing thermodynamic conclusions.

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THE FUNCTION OF PHOSPHATE IN ENZYMATIC SYNTHESSES

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INTRODUCTION

In this paper an attempt will be made to give a coherent picture of what we know about the function of phosphate in the thermodynamics of biological systems. If it is possible today to draw a complete picture and correlate and harmonize all the facts which we have accumulated—and I think it is possible to do so—we can attribute it to the fact that the material furnished originates from so many diversified quarters, some having a more physical, others a more chemical background. I may illustrate this with some examples.

Determination of heat changes accompanying trans- and dephosphorylations originated from Meyerhof's laboratory.¹ Combustion data of sugars have been collected by Neuberg and his group² in Germany and by the California school in this country. The latter school, represented by Parks and Huffman,³ Borsook⁴ and others, has furthermore been able to give us exact entropy values for a number of important biological compounds. The determination of biological oxidation reduction potentials, which was developed by Mansfield Clark and Michaelis, and their pupils, has also been of indispensable value for our understanding of energy relationships in biological systems. Finally, I ought to mention the long series of discoveries of new phosphoric esters, starting with the sugar phosphoric esters of Harden and Robison, followed in 1927 by the discovery of phosphocreatine by Fiske and Subbarrow, and ending with the isolation of acylphosphates by Warburg, Negelein and Lipmann in 1939.

THE POTENTIAL ENERGY OF TRIOSES

It is not the idea in this lecture to give a historical development of phosphorylation, but rather to illustrate that phosphorylations are processes of essential significance for the generation of energy in the living cell. We all know that the degradation of sugars is one of the most important energy-yielding reactions which take place in biological systems. It ought to be pointed out, however, that the general

formula $C_nH_{2n}O_n$ for sugars does not give us any information whatsoever about the potential energy of a compound. For instance, triose and lactic acid have both the formula $C_3H_6O_3$ but, as we shall see, the former possesses a much higher potential energy than the latter. Lactic acid is an α -hydroxy acid of relatively high stability, triose is a carbonyl-di-hydroxy structure of an extraordinarily high degree of instability. The two thermodynamically instable groups of triose are the carbonyl group, which can be oxidized to the very stable carboxylate group, and the dihydroxy structure, which by an anhydride reaction can be converted to the much more stable α -keto structure.

Before entering into a discussion of the thermodynamical role of phosphate, I think it is worth while for a moment to leave the phosphate out of the picture and see what would happen to the potential energy of the triose if this substance were converted to free pyruvic acid directly. The step $\text{glyceraldehyde} + \text{H}_2\text{O} \rightarrow \text{glycerate}^- + 2\text{H}^+ + 3\text{E}$ has already been discussed by Dr. Meyerhof, so only a few remarks are necessary. Although this step is only slightly exergonic at pH 0, i.e., when it is calculated from heats of formation under standard conditions, it is one of the strongest exergonic reactions known to occur in biological systems at pH 7. We know that when 2 hydrogen ions are liberated per 2 electrons the normal potential changes 60 millivolts (mv.) per pH unit. In this case, however, a third hydrogen ion is liberated, due to the formation of a carboxyl group. The pK of glyceric acid is around 3.4. At a more alkaline pH the acid is therefore practically completely dissociated and, consequently, the third hydrogen ion only affects the pH slope of the oxidation reduction potential between pH 0 and pH 3.5. This means that between pH 3.4 and pH 7 the oxidation reduction potential (E'_0) changes 90 mv. per pH unit, or, altogether 324 mv., and between pH 0 and pH 3.4, four times 60 mv. or 204 mv. The total change is therefore 528 mv. or approximately —25,000 calories. Since the conversion of $\text{glyceraldehyde} + \text{H}_2\text{O}$ to glycerate at pH 0 has a ΔF of —4000 calories,⁵ the ΔF of this reaction at pH 7 is —29,000 calories and at pH 8, around —33,000 calories. Very few biological oxidations with the exception only of other carbonyl oxidations do release such large amounts of free energy. It may be profitable to compare the ΔF of the reaction with that of other biological oxidations. The system $\text{lactate} \rightleftharpoons \text{pyruvate} + 2\text{H}^+ + 2\text{E}$ has, according to Barron and Hastings,⁶ an E'_0 at pH 7 of —180 mv. which corresponds to —8300 calories. Ball⁷ found the E'_0 at pH 7 of the pyridine nucleotide system to be —280 mv. which corresponds to —12,500 calories, i.e., still more than 15,000 calories short of the glycer-

aldehyde-glycerate-system, a fact of considerable importance as will appear later. The very high relative stability of the carboxylate group will also be discussed later.

The other important energy-yielding step, the formation of the anhydride pyruvic acid from the dihydroxy acid, glyceric acid, has recently been discussed by Lipmann⁸ in an interesting analysis. Lipmann has collected thermal data from various sources and has shown that the dehydration of glycerate to pyruvate liberates as much as 8000 calories per mole. Almost 2000 calories of this amount are derived from an increase in entropy due to the formation of a C=O group. The corresponding conversion of glyceraldehyde to methylglyoxal must give rise to approximately the same increase in entropy since the same structural changes are involved. The ΔH based on experimental combustion data, however, seems to be considerably smaller. The heat of combustion of methylglyoxal is, according to the careful determinations of Neuberg and Hofmann,⁹ 345,700 calories per mole; that of dihydroxy-acetone, according to Kobel and Roth,¹⁰ 343,100 calories. The heat of combustion of glyceraldehyde is presumably very nearly the same or, if anything, a little higher. The value, 338,000 calories, found by Neuberg, Hofmann and Kobel² applies only to the di-molecular solid glyceraldehyde. The mono-molecular glyceraldehyde has probably a combustion heat approximating 345,000 calories, judging from the equilibrium constant of the reaction phospho-glyceraldehyde \rightleftharpoons phospho-dihydroxyacetone.¹¹ Thus, the ΔF of the reaction glyceraldehyde — $H_2O \rightarrow$ methylglyoxal cannot be larger than — 2000 calories at most. It may also be of interest to mention, at this point, that the calculated heat of combustion ad modum Kharasch¹² does not check at all with the observed heats of combustion of methylglyoxal and dihydroxyacetone, but yields values 8000 to 15,000 calories too high.

The oxidation of methylglyoxal (α -keto aldehyde) to pyruvic acid (α -keto acid) has a ΔF of about — 27,000 calories at pH 7. If the free energy released by converting triose into pyruvate or lactate were simply scattered as heat, the conversion could follow two different pathways. The triose could first be dehydrated and then oxidized, or vice versa. The first pathway is that proposed by Neuberg:¹³ the anhydride of triose, methylglyoxal, is first formed, and this compound is then subsequently oxidized to pyruvate, or, by an internal dismutation, converted into lactate. Formation of methylglyoxal from triose is a non-enzymatic reaction, whereas conversion of methylglyoxal to lactate is catalyzed by a specific enzyme, the so-called methylglyoxa-

lase.¹⁴ Formation of pyruvate and lactate from triose through methylglyoxal may be a pathway of biological significance, although it is, for the time being, considered a process of no biological importance.

The second pathway, the study of which was initiated by Embden¹⁵ and continued by Meyerhof and Lohmann, seems to be the most important biological process for the degradation of sugar. The triose is first oxidized to glycerate and the glycerate is then dehydrated to pyruvic acid, which subsequently oxidizes another molecular of triose, itself being reduced to lactate. Whereas lactic acid can be formed from triose through methylglyoxal in the absence of phosphate, the pathway via glyceric and pyruvic acids requires phosphate and phosphorylated products.

Time will not permit me to go into any detail as to why the Embden scheme has been adopted as the basis for our concept of fermentation. The chemical synthesis of the phosphotrioses by Fischer and Baer,¹⁶ which made large scale experimentation with these compounds possible, had an important bearing upon the establishment of that scheme. The enzymatic studies of Meyerhof and Kiessling¹⁷ finally consolidated the Embden scheme.

THE RELATION OF PHOSPHORYLATIONS TO THE PRESERVATION OF POTENTIAL ENERGY

When we discuss the role of phosphate in carbohydrate metabolism, it is important to distinguish between the first steps in sugar oxidation (steps which are common to those of fermentation) and the further steps of oxidation (and decarboxylation) of the acids formed from the sugar acids.

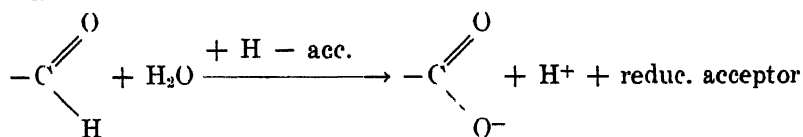
If rather special types of glucose oxidation are excluded, e.g., oxidation by glucose oxidase or by Robison ester oxidase, we shall see that phosphate plays a double role, so to speak, in the conversion of sugar to pyruvic acid. That is, only phosphorylated sugars are metabolized, and inorganic phosphate is necessary for the oxidation of the phosphorylated sugar.

The requirement of inorganic phosphate for various carbonyl oxidations, as well as the fact that only phosphorylated sugars participate in a number of important reactions, are features of major interest in a discussion of thermodynamics of biological phenomena.

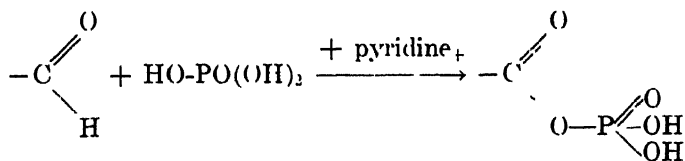
The coupling of fermentation with uptake of phosphate was first observed by Harden¹⁸ and Young, who also were the first to discover the formation of sugar phosphate esters. The thermodynamical impor-

tance of this coupling in connection with phosphorylation of creatine was first stressed in 1930 by Lundsgaard¹⁹ in his well-known studies of the anaerobic phosphocreatine breakdown during alactacid muscle contraction. Lundsgaard demonstrated for the first time a proportionality between the breakdown of a labile phosphate compound and mechanical work, and a resynthesis of the labile phosphate compound (phosphocreatine) coupled with lactic acid formation. The coupling between oxygen uptake and phosphorylation appears from the same studies, and was further strengthened by extract experiments of Dische,²⁰ Engelhardt, Meyerhof, and many others. The studies of Green, Needham and Dewan²¹ as well as of Meyerhof²² and his group showed that the coupling between the oxidation of phosphotriose and phosphate uptake is compulsory and mutual, and that the reverse reaction, the reduction of phosphoglycerate to phosphotriose, is coupled with a liberation of inorganic phosphate derived, as shown by Meyerhof, from adenosine triphosphate.

The mechanism of the coupling was explained by Warburg and his group through a number of most decisive experiments. Warburg and Christian²³ isolated and crystallized the phospho-glyceraldehyde dehydrogenase, and their collaborators, Negelein and Brömel,²⁴ isolated, as the primary oxidation product, di-phospho-glyceric acid as the strychnine salt. Expressed in more general terms,—what Warburg and his associates had shown was that the aldehyde group in triose is not oxidized as aldehyde *hydrate*, as suggested by Wieland and Thunberg,



but as aldehyde *phosphate*,

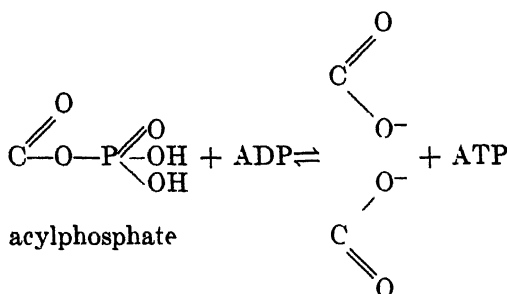


+ reduc. pyridine-nucl.

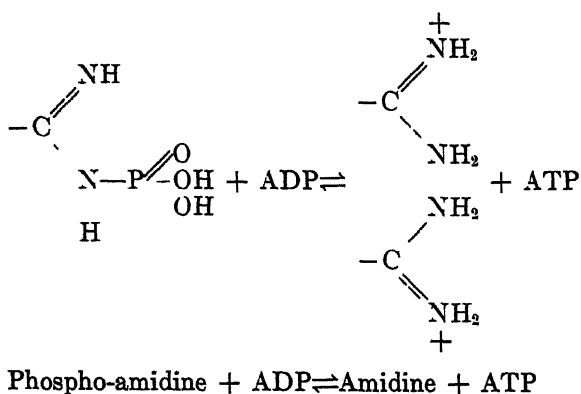
and this reaction is reversible due to the fact that instead of the stable carboxylate ion, the unstable acylphosphate, carboxylphosphate, is formed. Lipmann²⁵ has been able to show that the oxidation of pyruvic

acid to CO_2 and acetate follows the same pattern, at least in micro-organisms. Phosphate is necessary, and Lipmann²⁶ has recently succeeded in isolating acetylphosphate, the primary oxidation product. His most recent experiments indicate that also this reaction may be reversed.

Bücher,²⁷ in Warburg's laboratory, has isolated and crystallized another enzyme which catalyzes the equilibrium reaction: Diphosphoglyceric acid + adenosine diphosphate \rightleftharpoons Monophosphoglycerate + adenosine triphosphate, or in more general terms: Acylphosphate + ADP \rightleftharpoons carboxylate + ATP.

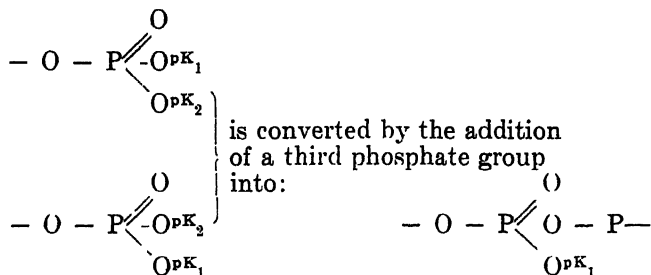


This equilibrium has an astonishing resemblance to that described by Lohmann, and later by Lehmann²⁸ in 1935:



Two mesomeric structures and a new pyrophosphate are formed in both cases. The same changes in degrees of mesomerism (resonance) apply to phosphate group No. 2 of adenosine diphosphate. This sec-

and group might be assumed to display a kind of mesomerism which could be pictured as follows:



It is a well-established fact that mesomerism, particularly of that symmetrical type described here, contributes to a great extent to the stability of a group.^{29, 30, 31} This applies, too, for free radicals, a fact which has been exploited so successfully by Michaelis.³² If a group of mesomeric character is phosphorylated, the symmetry disappears completely and thus the stability decreases greatly, which again means that the potential energy increases. The carboxylate group of phosphoglycerate or of acetate, the amidine group of creatine or arginine, the phosphate group of adenosine diphosphate, are all mesomeric structures which, upon phosphorylation, lose, more or less, their symmetry, and thus their mesomerism, and acquire instead what the structural chemists call "opposing resonance" (Coryell, cf.³³). All three types of phosphate esters just mentioned possess therefore a comparatively high potential energy. The important calorimetric studies of Meyerhof, Lohmann and Schulz give us an idea of the magnitude of the potential energy of these phosphate compounds. A determination of the ΔH of the splitting of phosphocreatine or adenosine triphosphate was undertaken soon after they were isolated by Fiske and Subbarow and was found to be 11,000–12,000 calories per mole P liberated.^{2, 34} The free energy of phosphocreatine and phosphoglycerate linkages must be close to that of the pyrophosphate in adenine nucleotides because of the readily reversed equilibria just mentioned.

The two pyrophosphate linkages of adenosine triphosphate must possess the same potential energy since the equilibrium constant of the reaction 2 adenosine diphosphate \rightleftharpoons 1 adenosine triphosphate + 1 adenylic acid⁴⁵ is very nearly 1. Thus, each of these linkages represents an amount of potential energy of at least 10,000 calories.

It was mentioned before that the ΔF going from carbonyl to carboxylate at pH 7 is — 29,000 calories. The ΔF of the reduction: pyridine-nucleotide \rightleftharpoons reduced pyridine nucleotide is approximately

+ 12,500 calories at pH 7. Thus, of the — 29,000 calories generated, only 12,500 are used for the reduction and the remaining — 16,500 calories are available for synthesis. Approximately —10,000 to 12,000 calories are stored either as acylphosphate, or more permanently as pyrophosphate or amidine phosphate energy. At pH 8 the energy balance is as follows: Reduction of pyridine nucleotide* + 13,750 calories; oxidation of glyceraldehyde — 33,000 calories. Thus, — 20,000 calories are available for synthesis at pH 8. In the back reaction the reduction of acid to aldehyde, which represents the most important and most expensive reaction in the synthesis of sugar from organic acids, the pyrophosphate linkage is sacrificed for the phosphorylation of the carboxylic group. The comparatively unstable acylphosphate is then readily reduced to carbonyl. The uptake of CO₂ in Lipmann's reaction may require more energy than the reduction of the carboxyl group of glyceric acid. However, Lipmann has recently reported that there are indications that also the oxidative carboxylations can be reversed.

Lipmann's discovery of acetylphosphate aroused a good deal of interest in other oxidative decarboxylations. Banga, Ochoa and Peters showed that phosphate is necessary for the oxidation of pyruvate in brain tissue. Ochoa³⁶ has recently found that phosphate is also required for the oxidation of α -ketoglutarate to succinate and CO₂. If we turn our attention to oxidations other than carbonyl oxidations, it appears that they also are able to furnish energy for phosphorylations. Thus, Colowick, Welch and Cori³⁷ showed that the step succinate-fumarate can give rise to phosphorylation of glucose. Moreover, oxidation of fumarate or malate gives rise under certain conditions, to formation of phospho-enol-pyruvate.³⁸ Whether phosphate is taken up by the fumaric acid forming phosphomalic acid, or whether malate or oxalacetate is phosphorylated by a phosphate donor is not known yet. The E'_0 of the malate-oxalacetate system is around — 180 mv., which means that if the flavine system transfers electrons the span between the potentials will amount to about 4500 calories.

If one compares the oxygen consumption with the uptake of phosphate, one is almost forced to assume that the higher oxidation steps too are coupled with uptake of phosphate. Ochoa,³⁹ as well as Belitzer and Tsihakova,⁴⁰ for instance, finds an average of 3 phosphate ester linkages per atom of oxygen, or 36 atoms of phosphate taken up per mole sugar, converted into CO₂. Dr. Ball has already mentioned the possibility that extra phosphorylation could be generated by the pas-

* This reduction involves two electrons but only one hydrogen ion.

sage of hydrogen from one co-enzyme to another, and has particularly called attention to the possible role of the alloxazine ring. The reduced alloxazine ring, particularly the C:O group in the 4-position, may be a point of entrance for the phosphate. In the oxidized alloxazine, the C:O groups form a conjugated pair of double bonds with the adjacent nitrogens. The mesomerism of these conjugated double bonds would cease to exist if phosphate groups were tied to the C:O groups.

If we now turn our interest to the phosphate esterified to the sugar, we realize soon that this problem has two aspects. The first is the phosphorylation of the alcohol group in the 6-position, which is of particular importance for the degradation of the dihydroxy structure; the other, the phosphorylation of the aldehyde group forming the 1-phosphoglucose or Cori ester, which is of importance for the formation of polysaccharide.

The fate of the alcohol phosphate ester linkage is of considerable interest in a discussion dealing with thermodynamics. Most important in this connection is the final conversion of the di-alcohol-ester into an enolic-ester linkage. Meyerhof and Lohmann⁴¹ found that one specific enzyme converts 3-phosphoglycerate into 2-phosphoglycerate where the phosphate group is on the carbon No. 2. They isolated another enzyme called enolase, which converts the 2-phosphoglycerate into the anhydride, phospho-enol pyruvate. They found, furthermore, that the latter ester has a high potential energy since it is capable of phosphorylating adenosine diphosphate to adenosine triphosphate. Correspondingly, the ΔH of the phosphate splitting is in the neighborhood of 10,000 calories.⁴² I have already mentioned the interesting calculations by Lipmann⁸ who showed that the dehydration from a dihydroxy structure to an α -keto structure is a strongly exergonic process. He calculated that ΔF of the reaction phospho-enol-pyruvate = pyruvate + phosphate amounts to 11,250 calories/mole.

The relatively high stability of the keto acid structure as compared to the dihydroxy structure must at least partly be ascribed to the tautomeric shift between the keto and the enolic structure. Just like the mesomeric oscillations are prevented by esterification, so is the tautomeric shift. Curiously enough, it has, so far, not been possible to demonstrate any phosphorylation of pyruvate by adenyl pyrophosphate. The reason for that remains obscure.

The role of phosphate in the synthesis of polysaccharides from monosaccharide has been revealed through the studies of Cori et al. They isolated in 1936,⁴³ as the primary product of polysaccharide splitting, a new glucose phosphate completely different from Robison ester, in

that it was extremely acid labile and that it had no reducing power. The structure of glucose-1-phosphate was established by Cori, Colowick and Cori⁴⁴ by synthesizing the ester. The formation of polysaccharide from 1-ester was indirectly observed by Schäffner, but decisively established by Cori, Cori and Schmidt⁴⁵ in this country and by Kiessling⁴⁶ in Germany. The recent studies of Cori, Cori and Green⁴⁷ further clarify several points, particularly why glycogen is necessary to initiate the reaction. Their formulation of the equilibrium is: glucose-1-phosphate + terminal glucose (of polysaccharide) \rightleftharpoons new maltosidic linkage + phosphate. Doudoroff⁴⁸ and his coworkers showed that the disaccharide, sucrose, is synthesized according to the same pattern. He found the ΔF of the sucrose synthesis to be + 1700 calories at pH 6.6. We meet here the same feature as we did when the carbonyl oxidations were discussed, namely, that a reaction which proceeds irreversibly, if water is involved, becomes reversible when water is replaced by phosphate. Thus, in the irreversible splitting of poly- or disaccharides by digestion enzymes, the glucosidic linkage is split by water; in the intracellular reversible splitting, the glucosidic linkage is broken by phosphate.

If we want to sum up very briefly what we actually have learned about the function of phosphate in biological syntheses, we may do it as follows. In the oxidation of sugars, the potential energy of the carbonyl group and the dihydroxy structure is transformed into energy rich phosphate linkages.

The next question is: What is the significance of the energy rich phosphate linkages? We know that adenylypyrophosphate is a powerful phosphorylating agent, particularly towards sugars and glycerol. Thus, adenylypyrophosphate initiates the carbohydrate metabolism, be it oxidative breakdown or polysaccharide formation, by phosphorylating hexoses. We have also seen how adenylypyrophosphate by phosphorylation of a carboxyl group can initiate the reduction of acids to aldehydes.^{21, 22} The existence of adenosine triphosphatase in myosin preparations, as described by Engelhardt and Ljubimowa, may be of significance for our understanding of how chemical energy is transformed into mechanical energy. However, only a vast extension of our knowledge of the chemistry of myosin will permit further conclusions in this direction.

ENERGETIC COUPLING WITH THE UPTAKE OF ALCOHOLS, SUGARS AND AMINO COMPOUNDS

I have not attempted, in this survey, to make a detailed account of all the numerous biological reactions in which phosphate is involved, but have rather presented the salient facts in the hope that they may provide some insight into the nature of coupled reactions in biological syntheses. This brings us to the question whether phosphate is involved in every biological synthesis or whether other compounds can react in a similar way. It is important to bear in mind that carbonyl groups can react to form more or less labile complexes with a large number of substances. If such a carbonyl complex were oxidized or polymerized we should have another example of a coupled synthesis. The model experiments of Baer⁴⁹ furnish particularly striking examples of such coupled syntheses. Baer found that the oxidation of α -keto compounds by lead tetra-acetate required the presence of hydroxyl furnishing groups such as water, phosphate or alcohols. When pyruvic acid was the substrate to be oxidized, and choline the alcohol furnishing the hydroxyl groups, the primary oxidation product was acetylcholine.

It is not unlikely that ammonia and amino groups can play a similar role as was described for hydroxyl groups. Thus, Annau⁵⁰ has reported that ammonia stimulates the oxygen consumption of tissue slices, and Örstöm⁵¹ found that the respiration of unfertilized eggs is greatly increased by addition of ammonium salts. There is every reason to be on the alert for the possibility that small amounts of ammonia or alcohols might be necessary components in certain enzymatic oxidations, particularly oxidations of carbonyl compounds. The amounts of alcohols or related substances necessary for such an oxidation might very well be so small as to escape our attention, since the same alcohol molecule might be used over and over again. We have an example of such a case in the action of minute amounts of arsenate on the oxidation of glyceraldehyde. According to Warburg and Christian⁵² arsenate works essentially in the same way as phosphate except that the corresponding carboxyl arsenate compound formed is so unstable that it breaks down at once, thereby liberating arsenate continuously. This explains why even traces of arsenate are able to maintain the oxidation of triose, whereas phosphate is required in much larger amounts. The finding of Warburg and Christian⁵² that glucose or fructose stimulated the oxidation of phosphohexonic acid may also be of interest in this connection. They found that the oxidation of phosphohexonic acid was

more complete in the presence of small amounts of the hexoses, although the latter were neither oxidized nor phosphorylated.

There is also evidence that esters other than phosphoric esters can serve as precursors in the formation of polysaccharides. This has been demonstrated most convincingly by Hehre and Sugg³¹ in their interesting studies of the bacterial synthesis of dextran from sucrose. They found that the reaction proceeds according to the following formula: glucose-1-fructose (= sucrose) + terminal glucose unit (of dextran) \rightleftharpoons fructose + new glucosidic linkage

Thus, the reaction is analogous to that discovered by Cori, differing only in that sucrose (which actually is glucose-1-fructose) takes the place of the Cori ester (glucose-1-phosphate).

There is reason to believe that reactions analogous to the enolase reaction (2-phosphoglycerate — $H_2O \rightleftharpoons$ phospho-enol-pyruvate) may exist. The conversions of cysteine or serine into pyruvate, reactions which have been studied by Smythe, Fromageot, Chargaff and Binkley, offer interesting examples of this type (cf. Binkley³⁴). I refer to Dr. Smythe's article³⁵ for further information in this regard. However, I should like to emphasize here one point of thermodynamical interest concerning the conversion of cysteine to pyruvate. If the change of free energy in this reaction should prove to be as large as that of the conversion of glycerate to pyruvate, it would imply that the removal of hydrogen sulphide from glutathione creates a powerful glycyl-glutamate donor, as the removal of water from phosphoglycerate forms a powerful phosphate donor.

It seems quite probable that the near future will witness the discovery of a number of important coupled reactions following the same pattern as those described for phosphoric esters.

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THE SIGNIFICANCE OF COUPLED REACTIONS FOR THE ENZYMATIC HYDROLYSIS AND SYNTHESIS OF PROTEINS

By

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Of the many problems which have excited the curiosity of the biochemist, there are few more challenging than that presented by the mechanism of the synthesis of proteins in living cells. The biochemical journals of the past two decades are replete with discussions of the probable reaction paths followed during the biosynthesis of proteins. In reviewing the salient literature concerning this question, it seems best to start with the generally accepted view that proteins consist largely of amino acid residues that are linked to each other by means of peptide bonds. This theory is based on the experimental finding that when proteins are hydrolyzed by proteolytic enzymes or by acids or bases, there are formed amino acids and amino and carboxyl groups are liberated. It is natural, therefore, that the question has been asked whether the biosynthesis of a protein molecule involves a reversion of the hydrolytic action of the proteolytic enzymes. In other words, does protein synthesis begin with the condensation of a number of amino acids to form peptide bonds?

The idea that the hydrolytic action of proteolytic enzymes might be reversed under certain experimental conditions is not a new one. Numerous investigators in the late nineteenth and early twentieth century observed that when proteolytic enzymes were added to concentrated solutions of the products of proteolysis, precipitates were frequently observed.¹ Later workers noted a decrease in amino nitrogen in the reaction mixture.² These results were taken to indicate that protein synthesis had occurred, although the evidence presented for the protein nature of the precipitates was unconvincing. The further study of this problem was energetically pursued by Wasteneys and Borsook in the 1920's. These investigators showed that when pepsin is allowed to act on egg albumin at pH 1.6 and the solution of the products of peptic hydrolysis is adjusted to pH 4 and concentrated, addition of pepsin results in the formation of a precipitate, designated plastein.³ This product was described as a protein largely on the basis of its digestibility by pepsin and its precipitation by trichloroacetic acid. There

has been considerable discussion in the literature concerning the protein nature of plastein. It may be sufficient to point out that the insolubility of plastein or its precipitability by trichloroacetic acid is not evidence of its protein nature; neither is the fact that it is digested by pepsin at pH 1.7. From the recent work of Flosdorf⁴ and others it seems probable that plastein represents a complex mixture of peptides of unknown chemical structure and that the average size of the individual peptides is rather small.

Of greater importance to this discussion is the question as to whether experiments performed under such complex conditions provide any evidence regarding the reversal of the hydrolytic action of proteolytic enzymes. In the first place, the formation of plastein gives no clear evidence of the formation of peptide bonds. Furthermore, the pH optimum for the so-called synthesis is quite different from that for the hydrolysis of proteins. In the absence of any evidence on this point, one may ask, therefore, whether there is any reason to believe that in the crude pepsin employed in the plastein experiments, there are different enzymes acting optimally on a given protein at various pH values. In that case, the synthesis of plastein might be effected at pH 4 by an enzyme or enzymes different from those that act hydrolytically at pH 1.6.

In discussing the theoretical aspects of plastein formation, it has been assumed, on the basis of the mass action principle, that one could apply the equilibrium equation

$$\frac{(\text{Products})^n}{\text{Protein}} = K$$

where n is the number of molecules of the hydrolysis products formed from a protein. On this assumption, increase of the concentration of the products should presumably favor synthesis. However, this picture is somewhat too simple because, as we shall see later, in the course of hydrolysis or synthesis in protein digests, a very complex sequence of reactions probably takes place in which the products of one reaction are in turn employed for a subsequent reaction. In the presence of a mixture of enzymes, some of which may not be proteolytic enzymes, some of these reactions may not involve the synthesis of peptide bonds at all. Such a complex situation scarcely warrants the introduction of the term "equilibrium constant." These remarks regarding the enzymatic formation of plastein do not dispute the idea that proteolytic enzymes can catalyze peptide synthesis; they are intended to indicate some of the uncertainties that arise when one attempts to study the

question of protein synthesis with substrates of unknown chemical nature.

At the present stage of development of our experimental methods, clearer information is gained by limiting the study of peptide synthesis to chemical systems much simpler than those encountered in concentrated protein digests. This means, in the first place, that it is necessary to prove unequivocally that proteolytic enzymes can link two amino acids together to form a peptide bond. This has been accomplished by selecting suitable amino acid derivatives and by the isolation and identification of the peptide synthesized by the enzyme. Through this technique it has been possible to effect the synthesis of single CO-NH linkages by each of the following enzymes: papain, bromelin, intracellular proteolytic enzymes of liver and spleen, and chymotrypsin.^{5, 6} Some of these enzymatic syntheses are presented in TABLE 1. These experiments give good evidence for the idea that pro-

TABLE 1

SYNTHESES BY PROTEOLYTIC ENZYMES

Enzyme: Papain

Benzoyl leucine + leucine anilide \rightarrow Benzoyl leucyl leucine anilide

Benzoyl phenylalanine + leucine anilide \rightarrow Benzoyl phenylalanyl leucine anilide

Carbobenzoxy phenylalanyl glycine + tyrosine amide \rightarrow

Carbobenzoxy phenylalanyl glycyl tyrosine amide

Enzyme: Chymotrypsin

Benzoyl tyrosine + leucine anilide \rightarrow Benzoyl tyrosyl leucine anilide

Benzoyl tyrosine + glycine anilide \rightarrow Benzoyl tyrosyl glycine anilide

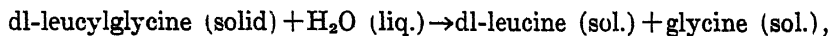
teolytic enzymes catalyze the attainment of equilibrium between a peptide and its split products and that, under suitable conditions, the enzymes can catalyze peptide synthesis.

Well-defined reactions of this type provide an opportunity of studying the enzymatic synthesis of peptide linkages with respect to the specificity of the enzyme action, the effect of pH and enzyme activators, and the manner in which peptide synthesis might be coupled with other reactions. We should like to outline some of the results to date and to speculate concerning the possible future directions of such investigations.

First, with respect to the specificity of peptide synthesis. If we assume that the action of a proteolytic enzyme is to catalyze the attain-

ment of equilibrium between a peptide and its hydrolytic products, then one should expect that the specificity of synthesis should be the same as that of hydrolysis. In other words, if we alter the chemical nature of one of the groups near a peptide bond in such a way that a given enzyme no longer is able to hydrolyze the bond, then a similar structural change in the components that are employed for the enzymatic synthesis will also prevent the formation of that peptide bond. For example, chymotrypsin requires for its hydrolytic action the presence, in its substrate, of the aromatic side chain of l-tyrosine or l-phenylalanine in the vicinity of the sensitive peptide bond. Replacement of this side chain with that of another amino acid such as leucine prevents hydrolysis.⁷ It even suffices to replace the l-tyrosine residue in benzoyl-l-tyrosylglycine amide by a d-tyrosine residue to render the resulting benzoyl-d-tyrosylglycine amide resistant to chymotrypsin action. Although the experimental material on the specificity of peptide synthesis is less extensive than that available for peptide hydrolysis, there is every reason to believe that the same precise specificity which applies to the hydrolytic action of chymotrypsin as well as other proteolytic enzymes also applies to their synthetic action.

Whatever the specificity of synthesis or hydrolysis of peptide bonds by a given proteinase may be, the reaction naturally cannot manifest itself if the thermodynamic prerequisites for its occurrence are not fulfilled. The hydrolysis of peptide linkages in proteins, as well as in simple peptides, proceeds spontaneously in the presence of a suitable enzyme. This indicates that the equilibrium which is established between hydrolysis and synthesis (of a peptide bond) is almost always very far on the side of hydrolysis. One must conclude that the hydrolysis of peptide linkages is a reaction that involves a decrease in the total free energy. An approximate idea of the magnitude of the free energy change involved in this hydrolytic reaction may be gleaned from the thermal data of Borsook and Huffman.⁸ According to their calculation, the reaction:



will result in the liberation of approximately 3000 calories. It is also stated that determinations of thermal data on other peptides indicate that the free energy changes in the hydrolysis of other peptide bonds are of a similar order of magnitude.

From these considerations it follows that the process of peptide synthesis in homogeneous solution requires, for its occurrence, energy which must be supplied by another reaction. Thermodynamic data

alone cannot tell us anything except the approximate amount of energy which will have to be introduced in order to make the total free energy change in the system negative. We must therefore look for the specific physical or chemical mechanisms that make possible the synthesis of peptide bonds.

Let us take the case of the peptide syntheses in the model experiments presented in TABLE 1. These syntheses occur because the products in all cases are rather sparingly soluble compounds. In the enzyme catalyzed reaction, therefore, the solution becomes super-saturated with respect to the synthetic product which then crystallizes from the solution. This crystallization disturbs the equilibrium and consequently more of the synthetic product is formed. The reaction thus proceeds in the direction of synthesis until the equilibrium ratio of concentrations has been established. In these peptide syntheses, therefore, the energy required for peptide synthesis comes from the process of crystallization whereby the synthetic product is removed from the equilibrium.

For example, benzoyl-tyrosyl-glycine-amide is specifically split by chymotrypsin to form benzoyl-tyrosine and glycine-amide. The hydrolysis is nearly 100 per cent complete if allowed to proceed for a sufficient length of time. If we try to reverse the hydrolytic process by allowing chymotrypsin to act on benzoyl-tyrosine and glycine-amide, we find that no appreciable synthesis has occurred. If we take glycine-anilide in place of glycine-amide, synthesis occurs and the expected product benzoyl-tyrosyl-glycine-anilide crystallizes out of the solution. The difference in the results with glycine-amide and glycine-anilide depends on the fact that the benzoyl-tyrosyl-glycine-amide is considerably more soluble than is the anilide. Under the experimental conditions of the enzyme experiment, the concentration of the amide at equilibrium is apparently much lower than that of a saturated solution of this compound. On the other hand, a saturated solution of the anilide is always at a lower concentration than the equilibrium concentration and as soon as a slight amount of the synthetic product is formed it is removed from the solution of crystallization.

Although the compounds employed in this model experiment are somewhat unphysiological, the mechanism which provides the energy for the peptide synthesis is not as unphysiological as might appear at first glance. We know that certain proteins are rather insoluble: collagen and elastin are two examples. It does not seem impossible that, in such cases, the energy for peptide synthesis may be provided, at least in part, by the removal of an insoluble synthetic product from the equilibrium. In general, we can say that any polyphase system which

involves the removal of the synthetic product from the enzymatic equilibrium will favor peptide synthesis.

Another mechanism for favoring the formation of peptide bonds is to couple the synthetic reaction with another energy-yielding chemical reaction which removes the synthesized peptide from the equilibrium with its precursors. An example of such a coupled reaction is provided by the following model experiment:⁹ The compounds, acetyl-dl-phenylalanyl-glycine and glycyl-l-leucine, when added separately to the proteolytic enzymes in papain, are not affected by the enzymes. However, when these two peptides are mixed and added to papain in aqueous solution, the glycyl leucine is hydrolyzed. This hydrolysis is accomplished through a series of three reactions. The first step involves a synthetic reaction in which the two peptides are joined to form acetyl-phenylalanyl-glycyl-glycyl-leucine. The driving force for this synthesis is provided by the subsequent enzymatic hydrolysis of the acetyl tetrapeptide. First a leucine residue is split off and then a glycine residue is removed. The compound acetyl-phenylalanyl-glycine, which is unchanged by this process, is termed a co-substrate.

It has been pointed out by Petrie,¹⁰ with some justification, that this is no way to synthesize proteins, since the final result of our efforts in this model experiment is a hydrolysis rather than a synthesis. Nevertheless, this model experiment serves as additional evidence for the conclusion that proteolytic enzymes are capable of catalyzing the synthesis of peptide linkages if the synthesis is coupled to another energy-yielding reaction.

There is another aspect of this coupled synthesis and hydrolysis which deserves attention. In the experiment discussed above, two peptides are combined by an enzyme with the subsequent hydrolysis of two peptide linkages. Under physiological conditions, proteolytic enzymes will frequently have at their disposal a number of peptides, as, for example, in the course of the enzymatic hydrolysis of a protein. In such cases, we must now face the situation that a complex series of coupled reactions may ensue in which there occur both synthesis and hydrolysis of peptide bonds. The course of this sequence of coupled reactions will depend on the specificities of the enzymes that are involved and the concentration and the chemical constitution of the peptides that are present.

It follows, therefore, that the digestion of a protein by a mixture of proteolytic enzymes can no longer be visualized solely as a sequence of hydrolytic reactions. We must also take into account the possible occurrence of coupled reactions which involve synthetic processes as well.

A further consequence of the concept of co-substrate action arises when we consider the effect of the introduction of a foreign peptide or protein into a system containing several enzymes and peptides. If this foreign substance participates in the sequence of coupled reactions, its presence may actually alter the fate of the proteolytic system. Attention has been drawn to the possible significance of such co-substrate action in the biological action of foreign proteins, such as the viruses.¹¹

All that has gone before has concerned the experimentally established mechanisms which can provide the driving force for the reversal of the hydrolytic action of the proteolytic enzymes. We are not in a position to state that reversal of proteolysis is actually the process employed by biological systems for the synthesis of peptide bonds. However, we know of no other enzymatic processes that show the precise specificity that characterizes the catalytic action of the proteolytic enzymes. At the present stage of knowledge concerning the biosynthesis of proteins, perhaps the best one can do is to continue to gather experimental information regarding the various types of energetic coupling which will result in peptide synthesis in well-defined chemical reactions catalyzed by proteolytic enzymes. We are certainly unable to state which of the many conceivable coupled reactions might actually occur in biological systems. In view of the multiplicity of proteins and the almost infinite possibilities of variation of their chemical structure, it would be surprising if there were only one or even only a few energy-yielding mechanisms involved in protein synthesis.

Several energy-yielding mechanisms may be envisaged. For example, in the case of the more complex proteins, such as the nucleoproteins, the chromoproteins, or the phosphoproteins, part of the driving force for synthesis of peptide bonds may be provided by chemical reactions involved in the linkage of the non-protein moiety to the amino-acid complex. We know from the extensive data now available on the specificity of proteolytic enzymes that, frequently, minor changes in the side chains of a peptide will decisively alter the sensitivity of the peptide to enzyme action.⁵ Thus, benzoyl-lysine-amide is split by crystalline trypsin (FIGURE 1). If the epsilon amino group is acylated, however, the peptide bond becomes resistant to proteolytic action. Another case is that found by Posternak¹² who showed that a phosphorylated dipeptide isolated on partial degradation of casein, phosphoseryl-glutamic acid, is resistant to the action of the proteolytic enzymes of intestinal mucosa; however, when the phosphoric acid residue was split off by the action of phosphatase, the resulting phosphorus-free peptide was readily split. On the basis of what we already know, it seems rea-

sonable to infer that a similar effect would be noted for the synthesis of the peptide. One may ask, therefore, whether in the course of the biosynthesis of a casein molecule, part of the energy for the synthesis of peptide bonds involving serine residues will be provided through coupling with phosphorylation reactions. Since methods are now avail-

Influence of Side Chain Substitution
on Hydrolysis of Peptide Bonds

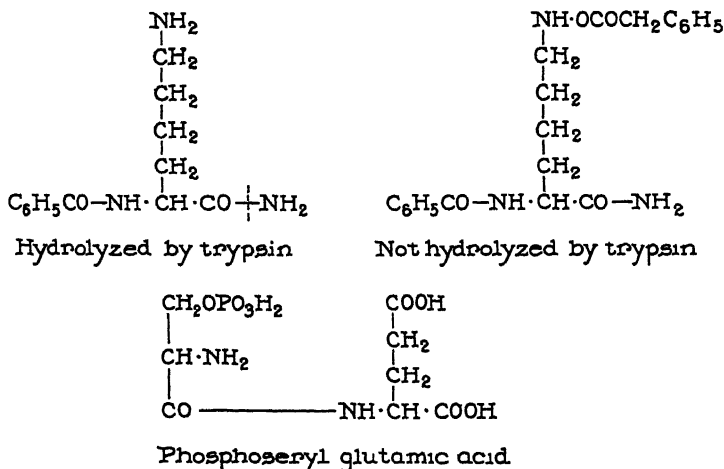


FIGURE 1.

able for the synthesis of peptides of l-serine,¹³ this possibility can be explored experimentally. Previous papers presented at this conference have discussed the role of phosphate in enzymatic syntheses. The desirability of further experiments on the relation of phosphorylation to peptide synthesis therefore needs no additional emphasis.

The epsilon amino group of lysine and the β -hydroxyl group of serine are only two of the many reactive groups in the side chains of naturally occurring peptides and proteins. If one considers the possibilities of reaction with the imidazole group of histidine, the sulfhydryl group of cysteine, or other reactive amino acid side chains, the possible variation in the nature of the energy-yielding chemical reactions involving the side chains of peptides becomes considerable indeed.

To these structural changes in peptides which may favor removal of the synthetic product from the equilibrium, there must be added the possible formation of intramolecular links between different parts of the peptide chain. In the specific case of keratin, which is resistant to the action of proteolytic enzymes, Goddard and Michaelis¹⁴ have shown

that this protein, on treatment with sulfhydryl compounds, is transformed into a product which is easily hydrolyzed by trypsin and pepsin. This change in behavior toward proteolytic enzymes has been attributed to the rupture of disulfide linkages in keratin. In addition to such disulfide linkages, other bonds have been suggested which might fix the peptide chains of proteins in certain spatial arrangements and thus cause these chains to undergo folding in a rather specific manner. However, the nature of the linkages that are involved in such folding of the peptide chain is not known, although this question has been the subject of extensive and stimulating speculation.

Another possible source of energy for peptide synthesis is, of course, the coupling with oxidation-reduction reactions. Although the view has been expressed frequently that such coupling exists, and it seems reasonable that it must be so, there is still no clear-cut experimental evidence on the subject. Maver and Voegtlin¹⁵ studied the effect of oxygen tension on the proteolysis of fibrin by papain. They concluded that proteolysis may be favored by the presence of sulfhydryl groups which on oxygenation may be converted into disulfide groups. It was assumed that the presence of these disulfide compounds favored protein synthesis. The difficulties that arise in the interpretation of these results are similar to those encountered in the discussion of the plastein formation in concentrated peptic digests. The complex mixture of peptides of unknown composition present in these oxygenation experiments makes it almost impossible to determine definitely the nature of the chemical reactions that are going on. Actually, as Linderstrom-Lang¹⁶ and Harris¹⁷ have shown, on oxygenation there may occur the formation of intermolecular disulfide linkages between peptides, thus giving rise to substances more insoluble than the parent substances. Mothes¹⁸ has also claimed a dependence of protein synthesis on oxygen supply, in this case for plants; however, here also the situation is unsettled and much further work is needed.

In the interpretation of these experiments of Voegtlin and Mothes, emphasis was placed on the essential role of sulfhydryl compounds in the activation of intracellular proteolytic enzymes. It was implied that by converting the sulfhydryl groups of a proteolytic enzyme into disulfide groups, the enzyme would cause peptide synthesis instead of hydrolysis. When this idea was tested in experiments with simple peptides of known structure, it was found that the same activation requirements were exhibited in the synthetic reaction as were found for the hydrolytic reaction.⁵ Whenever an intracellular enzyme required activation by HCN or cysteine for hydrolysis, similar activation was found to be

necessary for synthesis. If we view the activation process as the conversion of an inactive precursor into an active enzyme, then it would not be understandable from a thermodynamic point of view how the same enzyme could cause an equilibrium reaction to proceed in opposite directions depending solely on the state of activation of the enzyme.

Our knowledge of the mechanism of the activation of the intracellular proteolytic enzymes by HCN or sulfhydryl compounds is still incomplete. This is due, in large part, to the fact that they have not been purified to the same extent as have the enzymes of the digestive tract. At the present time, the experimental facts are best explained by the following picture of the activation process.¹⁹ An inactive α -proenzyme is converted by traces of reducing substances into a second β -form that is also proteolytically inactive (FIGURE 2). However, the second form

ACTIVATION OF INTRACELLULAR PROTEINASES

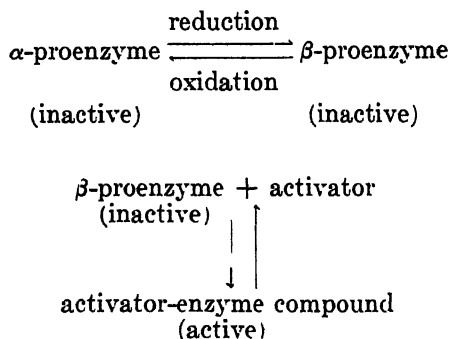


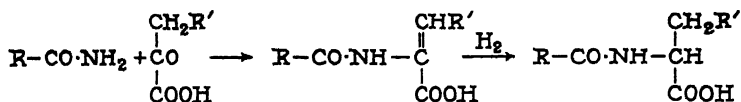
FIGURE 2

differs from the first in that it can combine with substances such as HCN, H₂S, cysteine or glutathione, to become an active proteolytic enzyme. The formation of the active enzyme requires relatively large concentrations of the activator. Not all compounds that are capable of forming addition compounds with the β -proenzyme can also convert the α -form to the β -form. An example of HCN which, even in high concentrations, cannot reduce the α -form to the β -form. If a trace of cysteine is added, however, the β -proenzyme is formed which, in turn, can combine with HCN to form an active enzyme. Of particular interest in this scheme is the fact that the enzyme-activator compounds that are formed are readily dissociable. If a volatile activator such as HCN or H₂S is employed, it is possible to inactivate the enzyme by simply removing the activator under reduced pressure.

It does not seem likely, however, that the intervention of oxidation-reduction reactions in the conversion of the α -proenzyme to the β -proenzyme will provide the requisite driving force of peptide synthesis by proteolytic enzymes. Thus far, no information is available concerning the precise nature of the biological oxidation-reduction systems that may be coupled with protein synthesis. Certainly, this problem presents one of the most interesting directions for future research.

Thus far, we have discussed the problem of the synthesis of peptide bonds in terms of the energetic coupling required to reverse the hydrolytic action of the proteolytic enzymes. As was indicated before, perhaps the strongest reason for believing that the enzyme catalyzed condensation of amino acids represents the most probable metabolic course of protein synthesis is the fact that the proteolytic enzymes, by virtue of their sharp specificity, are the only known biocatalysts which could direct, precisely and reproducibly, the complex sequence of successive peptide syntheses required for the formation of a protein. An additional reason for giving the proteolytic enzymes serious consideration in the scheme of protein synthesis emerges from the researches of Schoenheimer on the metabolism of isotopic amino acids. His work led him to conclude that, in the dynamic equilibrium between proteins and amino acids in the tissues, peptide bonds are constantly being broken and reformed under the catalytic influence of the tissue enzymes.²⁰

Nevertheless, in view of the present state of our knowledge, we cannot overlook the possible biological formation of peptide bonds by mechanisms other than the reversal of proteolysis. For example, it is known that acid amides can react with keto acids according to the following sequence of reactions to yield substituted dehydro amino acids.²¹



If an amino-acid amide would react in this manner a dehydropeptide would be formed. The coupling of this reaction with a reductive process would result in the formation of peptides. The existence of an enzyme apparatus required for such a series of reactions *in vivo* has not been demonstrated as yet, possibly because a systematic search has not been made. However, the biological occurrence of dehydropeptides is suggested by the presence, in animal tissues, of an enzyme system specifically adapted to the hydrolysis of dehydropeptides.²² As a

result of this enzymatic action, an amino acid, ammonia and a keto acid are formed. It seems of particular importance to investigate this enzyme reaction further and to determine whether, under suitable environmental conditions, it can be reversed. The recent developments in the synthetic methods for preparing dehydropolymers make available a large number of substrates for such enzyme experiments.²³

To continue this line of thought a bit farther, the important role of glutamine in animal metabolism, recently demonstrated by Van Slyke, Archibald and Hamilton,²⁴ raises the question whether this acid amide can react with keto acids with the resulting synthesis of CO-NH bonds involving the γ -carboxyl of glutamic acid. Of course, glutathione is the best known naturally occurring substance that has such a bond.

Still another chemical mechanism for the formation of peptide bonds is provided by the series of reaction shown in FIGURE 3 in which a

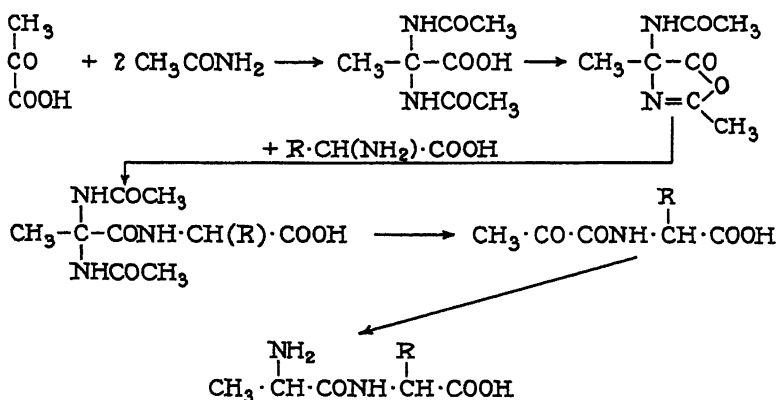


FIGURE 3. Formation of pyruvoyl amino acids and dipeptides.

pyruvoyl amino acid is formed.²⁵ Herbst and Shemin²⁶ have recently converted pyruvoyl alanine to the oxime, which on catalytic reduction yielded alanylalanine. The dipeptide could also be prepared from the ketoamide by catalytic hydrogenation in the presence of ammonia, according to the method of Knoop. A third method for converting the ketoamide to the dipeptide has been suggested by Linderstrom-Lang²⁷ and Agren.²⁸ According to this scheme, the ketoamide becomes aminated by a transamination reaction with glutamic acid. The complete Linderstrom-Lang scheme assumes the initial reaction of a ketoaldehyde such as methyl glyoxal with an amino acid, followed by an oxidation to the ketoamide.

These considerations concerning the possible chemical mechanisms for peptide synthesis are based partly on *in vitro* experiments and partly on speculation. The value of suitably chosen *in vitro* experiments for the study of chemical reactions *in vivo* is well illustrated by the history of our knowledge concerning the metabolic fate of arginine and its relation to creatine formation. In 1927 it was shown²⁹ that the series of chemical reactions shown in FIGURE 4 could be made to

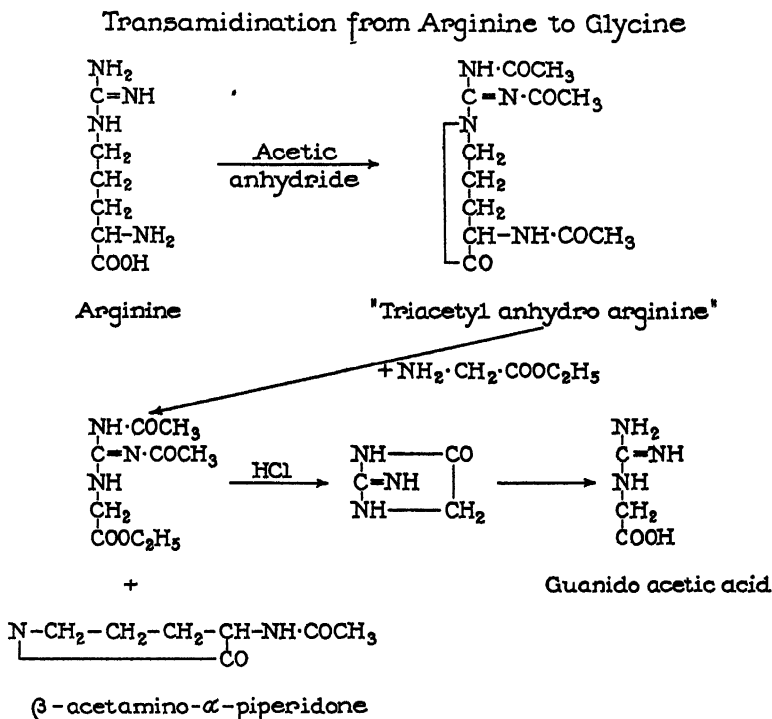


FIGURE 4.

occur. In essence, they represent the transfer of an amidine group from arginine to glycine. A similar transfer was also effected *in vitro* from arginine to sarcosine leading to creatine. These experiments drew attention to the possible biological transfer of an amidine group from arginine to glycine. The recent experiments of Borsook³⁰ and Schoenheimer³¹ have actually shown the biological occurrence of this process.

From the experimental results and the speculative considerations which have been presented in this paper, it follows that future progress

on the problem of the biosynthesis of proteins and peptides depends on a more intensive investigation of coupled reactions with well-defined enzyme systems and with substrates of known structure. Although the synthetic methods for preparing amino acid derivatives and peptides are far advanced, the purification of the tissue enzymes involved in peptide synthesis has lagged behind. We may be confident that, when the requisite intracellular enzymes are available in pure form, further progress in our knowledge of coupled proteolytic reactions will be forthcoming.

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SOME ENZYME REACTIONS OF SULFUR COMPOUNDS AND THEIR POSSIBLE INTERRELATIONSHIP

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Present information indicates that the three compounds, methionine, thiamine and biotin can supply the sulfur requirements of the mammalian organism under normal conditions. Methionine contains its sulfur as a thioether. Thiamine contains a thiazole ring and biotin contains a thiophene ring. The fact that these three compounds are required indicates that they are not interconvertible. Actually, a considerable amount of the sulfur requirement is supplied by the amino acid cysteine. It contains its sulfur in the sulfhydryl form. Thiamine may also exist to some extent in the sulfhydryl form. The thiazole ring opens rather easily under some conditions, forming a sulfhydryl group.^{1, 2}

A striking property of the sulfhydryl groups is that they are easily oxidized with the formation of disulfides. The disulfide corresponding to the sulfhydryl form of thiamine has been isolated and shown to retain its vitamin activity.³ The vitamin may actually function as a sulfhydryl compound. The disulfide of cysteine is, of course, the well-known cystine. All exclusively peptide compounds of cysteine retain a free sulfhydryl group, and they are free to undergo oxidation to the disulfide except in so far as steric factors prevent it. It is then a matter of some interest to know at what potential level the oxidation can occur for different sulfhydryl compounds. The potential level of the cysteine-cystine system has been the subject of considerable work. As is well known, cysteine and cystine, as we usually know them, do not behave in a thermodynamically reversible manner at a noble metal electrode. In many cases, the potential measured is independent of the cystine concentration.⁴ Perhaps the most obvious explanation of the system is in terms of Dr. Michaelis' free radicals. According to it, cystine is not the primary oxidation product of cysteine but is a stabilization product of the free radical formed by the oxidation (i.e., it is the dimer of the free radical). Since the disulfide bond of cystine is relatively stable, the concentration of free radical existing in equilibrium with it is too small to enable it to establish a potential at an electrode.

I think one might expect, from such a view, that different cysteine-containing compounds might vary in the stability of their disulfide form and, hence, they might impart a quite different potential to an electrode and might tend more toward thermodynamic reversibility. Thus, a protein sulfhydryl system might be more reversible than the cysteine-cystine system. In this connection, a comparison of the cysteine and glutathione systems is interesting. Some of the electrometric measurements indicated that the potentials established were about the same for different sulfhydryl compounds.⁵ The data of Fruton and Clarke⁶ on measurements of equilibria with dyes also indicated about the same value for cysteine and glutathione although their value was lower than the electrometric measurements. Dr. Rieser and I recently had occasion to test the reduction of both disulfide compounds polarigraphically. Although the method is not as simple as we might wish, it showed a distinct difference between the two compounds. We did not get the same result on oxidizing the SH compounds, but this behavior might be expected from available information. In a solution of pH 2.3 the midpoints of the reduction waves differed by 140 mv., the cystine being the more negative. In view of this result, we were interested to learn of the work of Rykkan and Schmidt⁷ at the University of California on the titration of these two compounds. They found that if cysteine was oxidized by iodine or iodate in the presence of excess iodide ion they got apparently normal titration curves. They got the same $E^{\circ'}$ values when cysteine and cystine were mixed in iodide containing solutions. Both methods showed an $E^{\circ'}$ for the system of + 0.27V. The $E^{\circ'}$ value was calculated according to the following equation:

$$E^{\circ'}_{(RSSR-RSH)pt} = E(Obn) + 0.283 - 0.059 \log \frac{(RSSR)^{\frac{1}{2}}}{(RSH)} + 0.059 \text{ pH.}$$

Other comparable data for glutathione indicate an $E^{\circ'}$ of + 0.45V. If such differences are real, then it would seem likely that SH groups in proteins may vary significantly in their normal potentials. The problem might well repay additional work.

Mammalian tissues contain only very small amounts of free cysteine or cystine, but all of them contain appreciable amounts of glutathione. A peculiarity in the structure of glutathione is that the glutamic acid is linked by the γ -carboxyl group. As far as we know, the glutathione structure does not exist preformed in protein but must be formed by some special enzyme or in some special manner. Our attention was recently directed to this problem in the following way. Dr. Neubeck and I had occasion to determine the total reducing material present in

guinea pig liver. To do so we deproteinized with metaphosphoric acid and titrated with iodine. We were surprised to find that the iodine titration increased rather rapidly as the time between the killing of the animal and the addition of metaphosphate increased. When this time was thirty minutes the titration was about 100 per cent greater than when the time was about two minutes. On checking into the matter, we found that much the same thing had been observed before and had been explained in different ways.^{8, 9, 10, 11} We were struck by the fact that the character of the end point changed with the increase in titration. Originally, it was sharp and definite as the end point with glutathione is under such conditions. Later, it was much less sharp and definite and resembled that with cysteine. It seemed to us that the in-

TABLE 1

Ratio glutathione/cysteine mls. I ₂ reduced	5:0	4:1	3:2	2:3	1:4	0:5
	5.65	7.41	9.65	11.62	13.43	14.03

creased iodine titration might be best explained, as Bierich and Rosenbohm¹¹ had suggested, by a hydrolysis of glutathione and liberation of cysteine. The figures in TABLE 1 show how the iodine titration, under such conditions, changes when the total SH is kept constant, but the ratio of glutathione to cysteine changes.

TABLE 2

FREE CYSTINE AND IODINE TITRATION VALUES OF DEPROTEINIZED EXTRACTS OF GUINEA PIG LIVER

Time After Removal of Liver from Animal (Minutes)	0	10	15	20	30	40	50	60
Animal 1								
Iodine titration*	12.5	16.1	17.1	21.6	22.6
Colorimetric cystine†	0.19	0.40	0.58	0.89	1.00
Animal 2								
Iodine titration	13.3	15.2	19.4	24.1	25.4
Colorimetric cystine	0.18	0.36	0.71	0.98	1.08
Animal 3								
Iodine titration	13.1	15.4	21.3	26.3	26.3
Colorimetric cystine	0.17	0.35	0.74	0.90	0.87
Animal 4								
Iodine titration	7.0	8.5	10.0	10.5	11.3	11.4
Colorimetric cystine	0.16	0.53	0.68	0.78	0.80	0.80
Animal 5								
Iodine titration	14.6	17.9	20.6	23.1	25.0	25.6
Colorimetric cystine	0.31	0.56	0.75	0.93	0.93	0.87

* Expressed as milliliters of 0.001M iodine per gram of tissue.

† Expressed as milligrams of cystine per gram of tissue.

In keeping with such an explanation was the fact that determinations by the Sullivan method¹² on successive preparations showed a definite increase in cystine paralleling the iodine titration (TABLE 2). Also, in keeping with the explanation, were the facts that the nitrogen of the deproteinized extracts prepared at successive intervals and the total cystine, determined after hydrolysis, did not change during the time that the iodine titration changed (TABLE 3). When more glutathione

TABLE 3

FREE CYSTINE, TOTAL CYSTINE AND KJELDAHL NITROGEN IN DEPROTEINIZED EXTRACTS OF GUINEA PIG LIVER

Minutes of incubation before deproteinization	0	13	23	33	43
Colorimetric cystine mg. per gram tissue	0.25	0.38	0.64	0.75	0.83
Total cystine after hydrolysis mg. per gram tissue	0.94	0.81	0.81	0.88	0.83
Kjeldahl nitrogen mg. per gram tissue	1.22	1.05	1.07	1.07	...

was added an additional change in titration and in cysteine occurred. In such cases, it was possible to isolate the cystine in good yield. From such findings, we conclude that the glutathione was being hydrolyzed and in amounts sufficient to account for the increased iodine titration.

From the fact that, in the liver fixed as rapidly as possible, the free cysteine is almost nil and the glutathione is quite appreciable (at least 2-3 mg. per gram), it is apparent that the equation representing the hydrolysis is pushed far to the glutathione side. In contrast, in the liver preparations, the glutathione is spontaneously hydrolyzed and the equilibrium reached corresponds to a large degree of hydrolysis. It seems to follow that, in the intact animal, the synthesis of liver glutathione must be coupled with an energy-yielding reaction.

It is quite likely that the enzyme concerned in this glutathione hydrolysis is the same as the one described originally as antiglyoxalase¹¹ and studied in more detail later.^{14, 15} For some reason, guinea pig liver is rich in the enzyme and rat liver is poor in it. According to our results, it was rather difficult to extract the enzyme from the liver. Much of the activity remained in the normally insoluble portion, but a solution with some activity could be obtained. The enzyme was found to have a rather alkaline pH optimum of 8.5-9.0 (FIGURE 1). Dialysis caused a prompt inactivation. If the dialysate was concentrated and readded to the non-dialyzable material, the activity returned. Similarly, a heated extract reactivated the dialyzed material and, rather in-

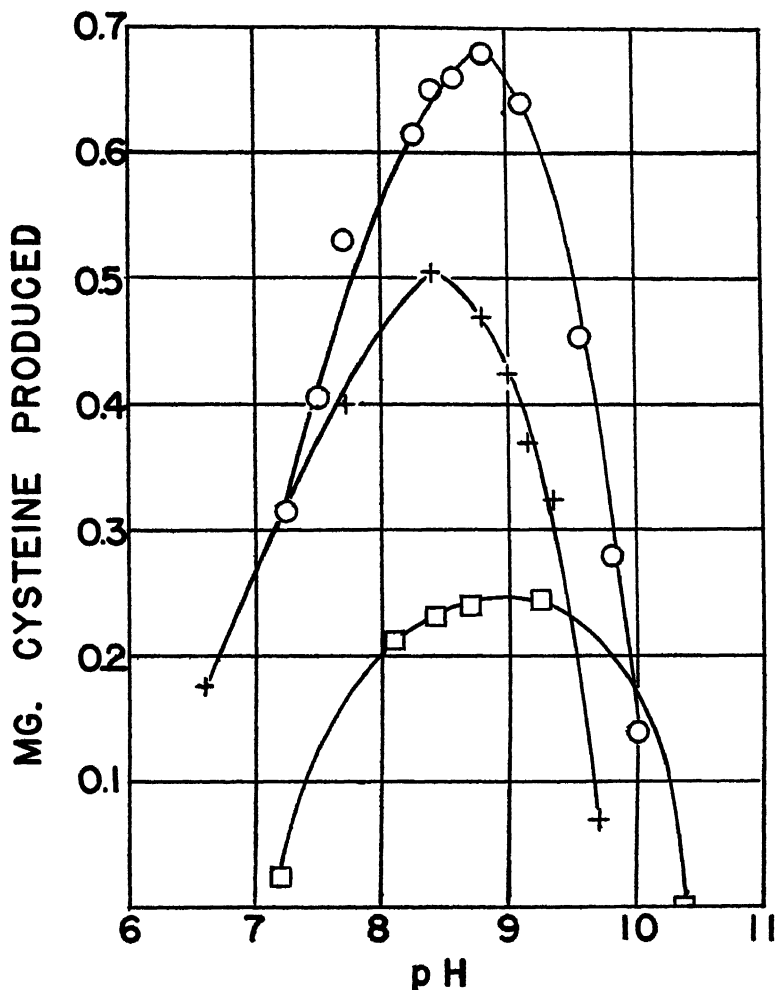


FIGURE 1. Optimum pH of Cysteine Production.

Production of cysteine by enzyme preparations as determined by the colorimetric method. The two upper curves indicate the production of cysteine by 20 ml. of guinea pig homogenate in 20 minutes, at 25° in the presence of 2.0 mg. glutathione in a total volume of 2.5 ml. The pH of the mixture was obtained with glycine buffer (x) and acetate-veronal buffer (o). The lower curve indicates the production of cysteine by 20 ml. of partially purified enzyme preparation in 30 minutes, at 25° in the presence of 2.0 mg. glutathione, in a total volume of 2.5 ml. Acetate-veronal buffer was used to adjust the pH of the mixture.

terestingly, an extract of rat liver that had only slight activity by itself readily activated the dialyzed enzyme from guinea pig liver. Apparently, rat liver contains the coenzyme but not much of the enzyme. The coenzyme is relatively stable. Irradiation with a mercury lamp for 2.5 hours did not inactivate it. Heating for one hour at 100° C.,

in either 0.5 N acid or 1.0 N alkali, caused only slight destruction. Wet ashing caused complete destruction. All of these results are included in TABLE 4.

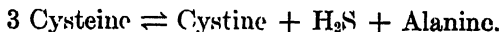
TABLE 4
RESTORATION OF ACTIVITY TO DIALYZED ENZYME MATERIAL

Material				Per Cent Activity
Original extract				100
"	"	dialyzed 20 hrs.		48
"	"	" 43 "		39
"	"	" 89 "		30
43 hr. dialyzed prep.	+	dialysate to orig. conc.		87
"	"	" + equal vol. heated extract		83
"	"	" + " 1.5 rat liver homogenate		78
89 hr. "	"	" + dialysate to orig. conc.		87
"	"	" + " irradiated 2.5 hrs.		
"	"	" + " at pH 8.4		96
"	"	" + " heated 1 hr. at 100° C. in		
"	"	" + " 0.5 m HCl		78
"	"	" + " heated 1 hr. at 100° C.		
"	"	" + " 1.0 m NaOH		74
"	"	" + " ashed		30

It is obvious that the organism makes some effort to keep glutathione intact and one may wonder what advantages it offers over the constituent amino acids. Of course, it may play a role in the synthesis of protein, but not much data is available on that. Only one specific function is known—that of coglyoxalase—and there is some doubt about how important a role the glyoxalase enzyme system plays. As far as the reducing properties are concerned, free cysteine contains the same reducing group, although, as stated above, the potentials are not the same. Of course, an obvious difference is that oxidized glutathione is more soluble than cystine, but another possible advantage is that glutathione may be more stable than cysteine to the action of some other enzymes present in tissues.

An example of such an enzyme is the one that produces H_2S from cysteine and has no effect on glutathione. The presence of such an enzyme in mammalian tissues was first observed by Fromageot and his coworkers in 1939.¹⁶ They observed the enzyme in dog liver and have since published several papers dealing with it. I first became acquainted with the enzyme largely by accident. We were studying the possible formation of ascorbic acid by rat tissues when it was observed.¹⁷

Fromageot and coworkers¹⁸ came to the conclusion that the reaction involved was

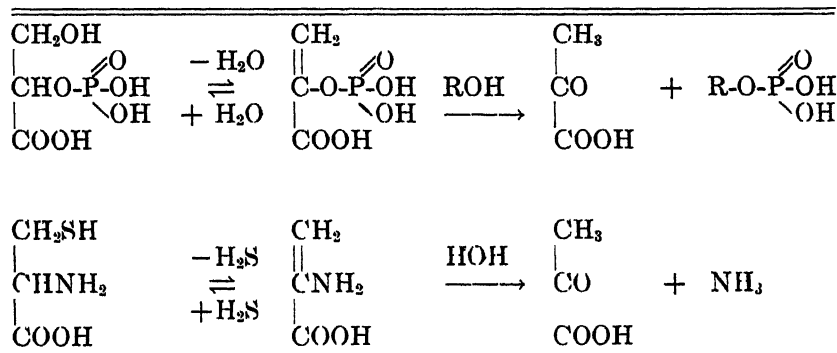


They emphasized that the enzyme was quite different from the enzyme in some bacteria that produces both H_2S and ammonia from cysteine.¹⁹ We were not able to confirm the above equation, but it may be correct to some extent. There does seem to be a tendency to form some cystine and small amounts of alanine. It might be possible to get conditions that would favor these products, but our results are much more in accord with the following equation¹⁷:



We were interested to see whether or not this H_2S formation could be reversed, so we allowed the reaction to occur in the presence of added H_2S containing radioactive sulfur.²⁰ After part of the cysteine had been converted the reaction was stopped and the cysteine present was isolated. It was found to contain appreciable amounts of radioactive sulfur. We consider this proof that sulfide sulfur was converted to cysteine sulfur and, since we do not know any other method of accomplishing this conversion, we will assume that the reaction that produces H_2S was reversed. All of our attempts to start with pyruvic acid, ammonia and H_2S , and demonstrate a formation of cysteine, were unsuccessful. These findings naturally suggest a comparison with the formation of pyruvic acid from 1-phosphoglyceric acid (TABLE 5). In this case, the

TABLE 5



first reaction is the removal of H_2O with the formation of phosphopyruvic acid and this reaction is readily reversible. The next step is the removal of phosphoric acid by a phosphate acceptor or by H_2O with the production of pyruvic acid, and this step is not reversible. A difference in the two cases is that in the phosphoglycerate case, the intermediate compound, phosphopyruvic acid, is stable enough to be

worked with, while in the cysteine case, the supposed intermediate, amino acrylic acid, is not stable. According to Chargaff and Sprinson²¹ and Binkley,²² serine undergoes a similar change and is converted to pyruvic acid via amino acrylic acid. Thus, pyruvic acid is produced enzymatically from three relatively different compounds by what appear to be quite similar reactions. Such findings are in very good agreement with the earlier work of Bergmann and coworkers²³ on the chemical behavior of such compounds. It has been suggested that the same enzyme may be acting on all three compounds.^{21, 22}

Some information is available on the partial purification and properties of the H_2S forming enzyme. It is inhibited by cyanide^{16, 17} by As_2O_3 ²³ and by such carbonyl reagents as phenylhydrazine, hydroxylamine and semicarbazide.²⁵ The activity of the crude tissue preparations is not inhibited by NaF ,^{16, 17} but the purified enzyme is inhibited.²² Some other amino acids do not inhibit, but other thiol compounds do.²⁵ Magnesium apparently forms a component part of the enzyme^{25, 22, 21} although it can be replaced by some other metals.²²

The question of a name for the H_2S forming enzyme is of some interest. Fromageot and coworkers called the bacterial enzyme that produces H_2S and NH_3 from cysteine, cysteinase; and they called the one in mammalian tissue that, according to them, produces H_2S but no NH_3 , desulfurase. It is doubtful if these enzymes differ, at least in the manner suggested, and hence two names are possibly too many. The term cysteinase is not very descriptive. The term cysteine desulfurase probably causes no confusion at the present time, but if the suggested mechanism of the reaction is correct the name would seem to be incorrect. It is at least theoretically possible to remove sulfur, as such, from cysteine and an enzyme that could do so would properly be a desulfurase. However, the fact that hydrogen is removed along with the sulfur is rather an important one, and, I think, should be indicated in the name if possible. Calling an enzyme that removes H_2S a desulfurase is analogous to calling an enzyme that removes H_2O a deoxygenase. If we call the addition of H_2O a hydration and an enzyme that removes H_2O a dehydrase then it would seem reasonable to call the addition of H_2S a sulfhydration, and an enzyme that removes H_2S desulfhydrase. The enzyme that produces H_2S from cysteine would then be cysteine desulfhydrase.

In view of the fact that H_2S is very toxic for an intact animal, it seemed curious that tissues should contain an enzyme for producing it. The possible connection of such H_2S formation with the known toxicity of cysteine for young white rats under certain conditions seemed ob-

vious. Actually, we could find no data on the effect of H_2S on isolated tissues, so we tested the effect of adding H_2S to liver, kidney and brain preparations.²⁶ It turned out that the oxygen consumption of liver and kidney was not at all sensitive to H_2S , while that of brain was quite sensitive. Apparently the toxicity of H_2S is not a general tissue phenomenon. Such a finding could be utilized in studying the enzyme reactions involved.

The fact that methionine is an essential amino acid and cannot be replaced by cystine is well established.²⁷ The fact that its methyl group can be removed and transferred to other compounds is also well established.²⁸ Apparently, the transmethylation is reversible, for methyl groups supplied as choline can be recovered in methionine.²⁹ The enzymes concerned in this reaction have not been studied in detail, as far as I know. The homocysteine resulting from the demethylation of methionine is another sulfhydryl compound. The addition of it to liver preparations results in the formation of some hydrogen sulfide.^{27, 30} According to my measurements, much less H_2S is produced from homocysteine than from cysteine, and I would normally assume that both compounds are probably acted upon by the same enzyme. However, Fromageot and Desnuelle³¹ report that the ratio of the activity on the two compounds is quite different for different enzyme preparations and they conclude that they are acted upon by different enzymes. The other products resulting from this action have not been determined.

Binkley and du Vigneaud³² have found that homocysteine plus serine, in the presence of liver extracts, produces cysteine. It is interesting that in this case also pyruvic acid plus ammonia will not replace serine. With my extracts, I was not able to replace homocysteine with H_2S . Binkley and du Vigneaud did not obtain cysteine when they used methionine instead of homocysteine, but Floyd and Medes³³ report that methionine added to liver or kidney slices, under aerobic conditions, results in cysteine formation. However, the amount of cysteine obtained was very small.

The sulfhydryl group can be oxidized further than the disulfide stage and can produce inorganic sulfate, or, in the case of cysteine, cysteic acid. Some tissues contain an enzyme that decarboxylates cysteic acid,^{34, 35} producing taurine. Thus, an enzymatic path is charted for the conversion of methionine to cysteine and to taurine, reactions that were well established on intact animals.

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